BRITISH JOURNAL OF CANCER

VOL. X JUNE, 1956 NO. 2

INTRA-EPITHELIAL CARCINOMA OF PROSTATIC URETHRA, PERI-URETHRAL GLANDS AND PROSTATIC DUCTS (" BOWEN'S DISEASE OF URINARY EPITHELIUM ")

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Received for publication March 7, 1956.

PAGET'S disease of the nipple is regarded by many workers as being due to the intra-epithelial spread of carcinoma cells from an underlying carcinoma of mammary ducts (Muir, 1927, 1939 ; Inglis, 1936, 1946) and the rare cases of extra-mammary Paget's disease in the peri-anal, vulval and axillary regions as a similar lesion associated with a carcinoma of underlying apocrine sweat glands (Weiner, 1937 ; Plachta and Speer, 1954 ; and many others). Paget's disease of the eyelid associated with a carcinoma of Moll's gland, which is probably a modified apocrine sweat gland, has also been reported (Whorton and Patterson, 1955).

Ortega, Whitmore and Murphy (1953), however, have reported a case of " *in situ* carcinoma of the prostate with intra-epithelial extension into the urethra and bladder " which they describe as an extracutaneous form of Paget's disease. In this case there were intra-epithelial groups of large bizarre hyperchromatic cells in the epithelium of the prostatic urethra extending into the ejaculatory ducts and the ducts and some acini of the prostate. Some of the intraprostatic ducts were filled by sheets of flattened " squamoid " malignant cells, but numerous histological sections showed no spread into the stroma through the duct walls, even though the cells extended down to the basement membrane. Clumps of similar cells were also found scattered throughout the bladder mucosa, and in one small vesical lesion there appeared to be some infiltration of the stroma.

Case report

We have seen a similar case in a man, aged 78 years, who had a sub-total prostatectomy for prostatic obstruction. He also had a large ulcerated tumour of bladder, biopsy of which showed a transitional cell carcinoma infiltrating muscle. This was treated by radon seed insertion, but the patient died with an extensive local recurrence one year after operation.

Pathological findings

The extravesical malignant lesion involved portions of the prostatic urethra (Fig. 1) and the peri-urethral glands, some of which were filled with cells resembling those described by Ortega, Whitmore and Murphy (1953) (Fig. 2 to 5) while others

showed irregular hyperplasia of transitional epithelium with some mitotic activity (Fig. 6, 7). A few prostatic ducts and acini near the urethra were also involved (Fig. 9, 10). The epithelium of the prostatic urethra also showed localised patches of irregular epithelium in which the normal arrangement of the cells was lost and the nuclei deeply staining and varying in shape and size. The cytoplasm in some cells was vacuolated (Fig. 11 to 13), forming a halo around a large hyperchromatic nucleus. The sub-epithelial connective tissue was congested and infiltrated with round cells. The picture here is that of Bowen's disease (Bowen, 1912; Stout, 1939) of the urinary tract, as described by Melicow and Hollowell (1952). The remainder of the prostatic tissue removed showed benign nodular hyperplasia. There was no invasion of the stroma by malignant epithelium. Some of the malignant cells in the peri-urethral glands and surface epithelium (but not the vacuolated "Bowen cells") contained P.A.S.-staining material which was not removed by salivary digestion (Fig. 5 and 13).

DISCUSSION

Melicow and Hollowell (1952), in a report on thirty cases of Bowen's disease of the urinary tract, describe three cases in which lesions were found in the prostatic urethra. One of these was also associated with a large infiltrating carcinoma of bladder. Although occurring in the prostate it seems likely that lesions of this type are probably due to a carcinogenic stimulus affecting transitional epithelium and are probably more closely related to the transitional cell tumours of the urinary tract.

A number of different names, depending on the anatomical site or minor changes in the clinical appearances, have been given to lesions with histologically similar appearances: Paget's disease, Queyrat's erythroplasia, Bowen's disease,

EXPLANATION OF PLATES

FIG. 1.—A group of large hyperchromatic cells with irregular nuclei in the epithelium of the prostatic urethra. H. & E. × 550.

FIG. 2.—Similar cells in a peri-urethral gland. H. & E. × 550.

FIG. 3.—A group of affected peri-urethral glands. H. & E. × 85.

FIG. 4.—Another group of affected peri-urethral glands; some of the cells lie in clear spaces. H. & E. × 170.

FIG. 5.—A section from the same block as the preceding figure, stained by the periodic acid-Schiff method. Some of the cells contain mucoid material (black), but the clear spaces seen in Fig. 4 do not have this appearance. × 170.

FIG. 6.—Irregular hyperplasia of transitional epithelium in some peri-urethral glands. H. & E. × 85.

FIG. 7.—A high power field from Fig. 6 showing nuclear irregularity. One cell is in mitosis. H. & E. × 1250.

FIG. 8.—An affected prostatic duct is seen at the top. One side is lined by normal columnar epithelium. H. & E. × 85.

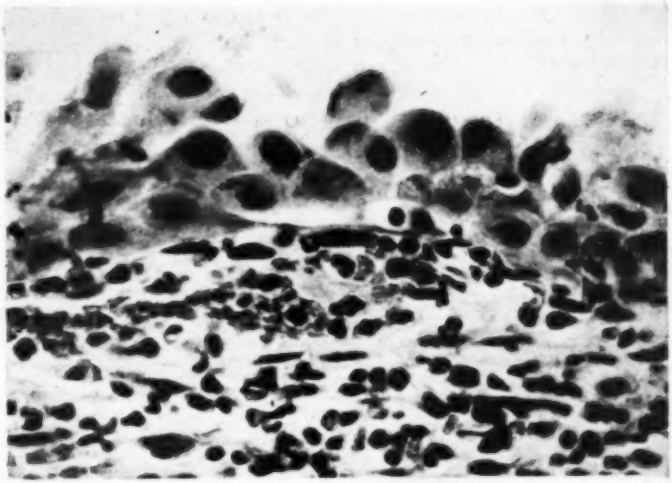
FIG 9.—An involved prostatic acinus. H. & E. × 100.

FIG. 10.—A high-power field from the area marked in Fig. 9. The hyperplastic cells are covered by the low columnar epithelium of the acinus. H. & E. × 550.

FIG. 11.—Two localised patches of thickened irregular epithelium in which vacuolated cells can just be made out. H. & E. × 75.

FIG. 12.—A high-power field from the plaque on the right, showing several clear "Bowen cells" in the epithelium. H. & E. × 410.

FIG. 13.—A similar area to Fig. 12. The superficial cells contain a little mucoid material but the Bowen cells do not. P.A.S. stain. × 410.



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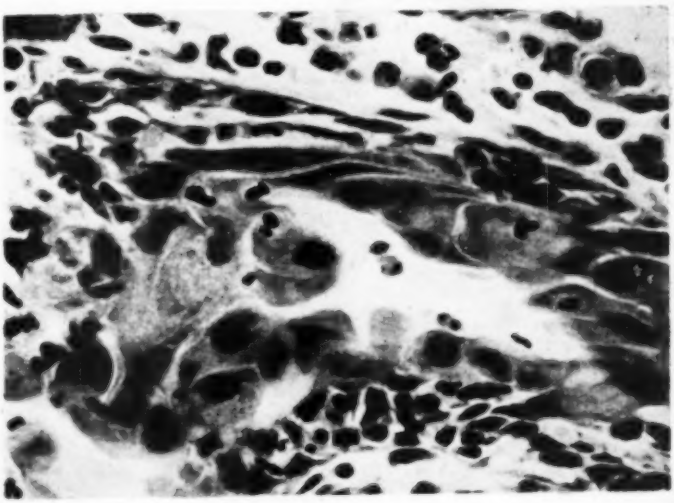

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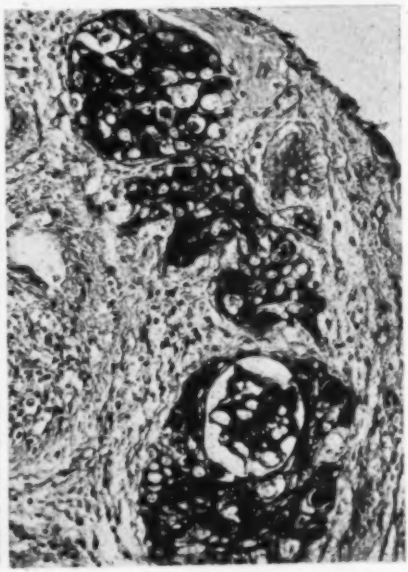

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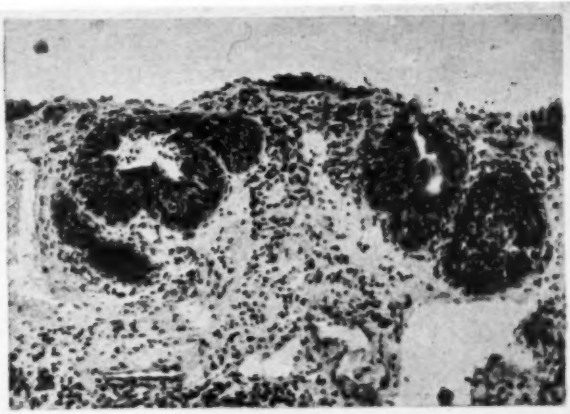

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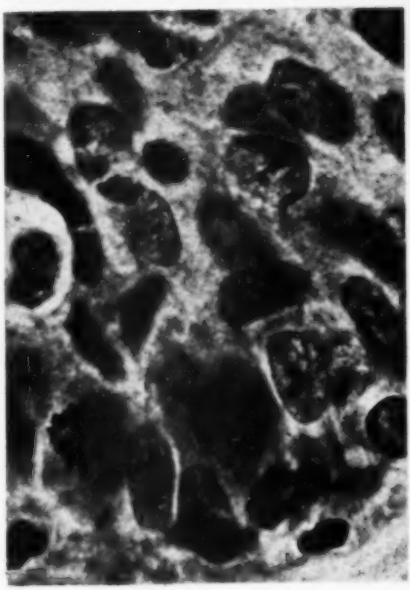

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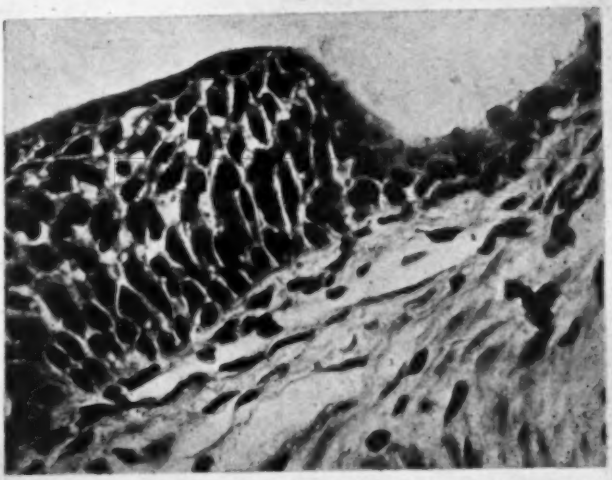

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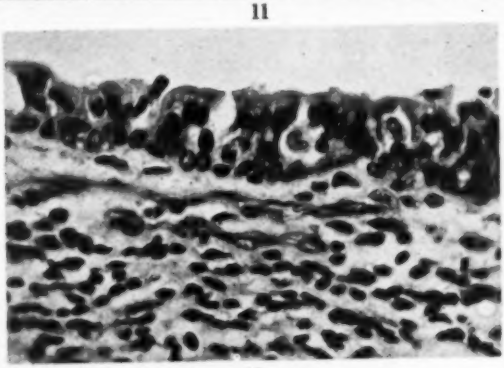

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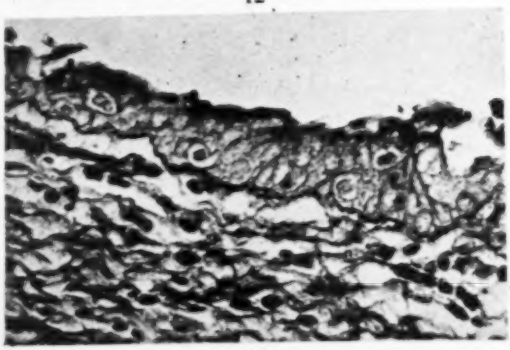

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carcinoma *in situ*, etc. These lesions are commonly found in the skin and less often in the mucous membranes (glans penis, lip, vulva, vagina, tongue, nares, uvula, vocal cord, palate, tonsils (Stout, 1939 ; Cipollaro and Foster, 1940) and urinary tract (Melicow and Hollowell, 1952)). The essential histological features in the skin, as described by many workers, and in the mucosal lesions are similar but the changes are often not so distinctive in the mucous membranes (Stout, 1939).

Paget's disease of the nipple can be separated from this group in that it is almost invariably associated with an adenocarcinoma of the underlying breast. In other sites it would seem preferable to restrict the term " Paget's disease " to epithelial lesions which are also associated with carcinoma of an underlying gland.

The other lesions in the group (Bowen's disease, etc.) may also be associated with infiltrating carcinomatous lesions, but in these cases the carcinomas are almost always squamous (Stout, 1939) or in the urinary tract, transitional celled (Melicow and Hollowell, 1952) and arise from the surface epithelium. One further point of interest is that mucosal Bowen's disease is much more frequently associated with invasive tumours (40 per cent of cases) as compared with the skin lesions (2 to 3 per cent) (Stout, 1939). Fennell and Castleman (1955) suggest that these lesions are cytologically malignant and should be considered as a form or stage of actual cancer (carcinoma *in situ* or pre-invasive cancer) rather than a pre-cancerous lesion.

SUMMARY

A case of intra-epithelial carcinoma of prostatic urethra, peri-urethral glands and prostatic ducts associated with a carcinoma of the bladder, is reported. If an eponymic title to this type of lesion is required, " Bowen's disease of urinary epithelium " is preferable to " Paget's disease of the prostate ", but a simple descriptive title, e.g. pre-invasive cancer or cancer *in situ* is preferable to either.

Our thanks are due to Dr. J. Bamforth, who originally suggested the diagnosis in this case.

REFERENCES

Bowen, J. T.—(1912) *J. cutan. Dis.*, **30**, 241.
Cipollaro, A. C. and Foster, P. D.—(1940) *N.Y. St. J. Med.*, **40**, 264.
Fennell, R. H., Jr., and Castleman, B.—(1955) *New Engl. J. Med.*, **252**, 985 and 1032.
Inglis, K.—(1936) ' Paget's Disease of the Nipple ; and its Relation to Surface Cancers and Precancerous States in General.' London (Oxford University Press).
Idem.—(1946) *Amer. J. Path.*, **22**, 1.
Melicow, M. M. and Hollowell, J. W.—(1952) *J. Urol.*, **68**, 763.
Muir, R.—(1927) *J. Path. Bact.*, **30**, 451.—(1939) *Ibid.*, **49**, 299.
Ortega, L. G., Whitmore, W. F. Jr., and Murphy, A. I.—(1953) *Cancer*, **6**, 898.
Plachta, A. and Speer, F. D.—(1954) *Ibid.*, **7**, 910.
Stout, A. P.—(1939) *N.Y. St. J. Med.*, **39**, 801.
Weiner, H. A.—(1937) *Amer. J. Cancer*, **31**, 373.
Whorton, C. M. and Patterson, J. B.—(1955) *Cancer*, **8**, 1009.

SQUAMOUS EPITHELIAL METAPLASIA IN THE RESPIRATORY TRACT IN URAEMICS

A PRELIMINARY REPORT ON A STUDY OF POST MORTEM CASES

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Received for publication February 23, 1956

By squamous epithelial metaplasia in the respiratory tract is meant a substitution of columnar cell epithelium by squamous epithelium, stratified in at least 3 distinct cell layers.

Changes of this nature have been observed in connection with both acute and chronic irritations of the respiratory tract (Askanazy, 1919; Niskanen, 1949; Wegelin, 1919; Weller, 1953; Weller, 1955, personal communication), and also in cases of vitamin A deficiency as part of a more general epithelial alteration.

Metaplasia in the respiratory tract is of particular interest as a possible precancerous condition.

In a systematic investigation of epithelium in the respiratory tract in over 200 autopsies, we were struck by unusually marked and widespread metaplastic changes in a young man belonging to an age group where metaplasia was otherwise rare. The patient died of uraemia following a subacute glomerulonephritis. There were no other known causes of epithelial metaplasia which offered a natural explanation of the changes observed in this case.

Our autopsy material was therefore examined with a view to metaplasia in uraemia.

In 45 cases under 20 years of age only six had metaplasia, and one of these six, the above mentioned young man (Case 1), died of uraemia caused by kidney disease.

The adult material is summarized in Table I. In 169 adults there were 44 men and 29 women with uraemia. There appeared to be some preponderance of metaplasia in this group of uraemics compared with its incidence in the total number of cases (Table I). The difference, however, was not significant. Several of these cases had had uraemia of short duration as a terminal stage of some other disease, such as circulatory failure, surgical abdominal diseases, or acute urinary retention.

The material was therefore scrutinized, and those cases of uraemia selected where there was definite clinical and pathological proof as well as laboratory evidence that some serious chronic kidney disease was the cause of the uraemia. Ten men and 7 women fell into this category. Metaplasia was found in the epithelium of the respiratory tract in all of these cases.

The ages of the individuals varied between 22 and 86 years, but several of those concerned were young persons. Different types of chronic kidney disease were the causes of uraemia (Table II).

TABLE I.—*Metaplasia of the Respiratory Tract Epithelium in Uraemic Cases, Compared with the Frequency of Metaplasia in Total Number of 169 Post Mortem Cases.*

In cases of chronic kidney disease uraemia, metaplasia was found in 100 per cent. "Grading of metaplasia" refers to the extent of the metaplastic change.

Grading of metaplasia.	Total material.		Uraemic cases.		Chronic kidney disease.	
	M.	F.	M.	F.	M.	F.
No metaplasia	30	39	12	14	0	0
Metaplasia Grade I	20	17	10	9	4	5
Metaplasia Grade II	17	9	6	3	1	2
Metaplasia Grade III	33	4	16	3	5	0
Total	100	69	44	29	10	7
% Metaplasia	70	43·5	73	51·7	100	100

TABLE II.—*Types of Chronic Kidney Disease Causing Uraemia.*

Diagnosis.	Number of cases.	
	Male.	Female.
Chronic cysto-pyelonephritis	6	2
Myelomatosis of the kidneys	1	—
Hydronephrosis	1*	2
Tuberculosis of the kidneys	1	2
Chronic glomerulo-nephritis	1†	—
Polycystic kidneys	—	1
Total	10	7

* Case 2. † Case 3.

It would appear from these findings that kidney disease with uraemia might in one way or another lead to squamous epithelial metaplasia in the respiratory tract.

In some of the patients, particularly the older ones, a number of other causative factors may have been active in the development of metaplasia, e.g. chronic irritation of the mucous membrane of the respiratory tract due to polluted air or chronic and acute disease of the respiratory tract. The case histories of the younger patients, however, do not indicate such additional causes.

In order to elucidate further the possible connection between uraemia and metaplasia of the respiratory tract epithelium, a subsidiary investigation of current autopsies was carried out over a period of two months.

We found 2 more cases where uraemia was the cause of death subsequent to kidney disease. These two were young women (Cases 4 and 5) both with considerable metaplastic changes of the bronchial epithelium (Fig. 3 and 4).

In our studies we have examined several microscopic slides from 11 different locations in the bronchial tree. In the subsequent case reports the metaplasia is graded according to the following criteria :

Grade I : Metaplasia in one location.

Grade II : Metaplasia in two or three locations.

Grade III : Metaplasia in four or more locations.

Case 1.

0.93/54. Seventeen-year-old schoolboy.

In October 1953 protein was found in the urine at a routine school examination. Last 2 months—gradual increasing dyspnoea and listlessness, which led to confinement to bed. Died 6 hours after admission to hospital in February 1954.

Autopsy revealed a slim, pale, young man. The kidneys were large (380 and and 360 g.) and showed microscopically the typical picture of a diffuse subacute glomerulonephritis.

Lungs and respiratory tract were almost normal in colour compared with the other very pale organs. In the lower part of the trachea and in the bronchi, small diffuse patches of very pronounced metaplastic changes of the epithelium were found, which had 8–10 distinct flat cell layers (Fig. 1 and 2). Grade III metaplasia.

Case 2.

0.470/54. Thirty-one-year-old dairyman.

In 1949 protein in the urine, and later repeated stays in hospital because of uraemia, the last time with haemoptysis, marked hypertension, and signs of myocardial infarction (E.C.G.) Died October 1954. Blood urea 414 mg. per cent. Autopsy revealed pale, oedematous organs with the exception of hyperaemic lungs.

Kidneys: Small granular; microscopically, pronounced changes showing chronic glomerulonephritis. Infarction in the anterior wall of the heart.

There were extensive metaplastic changes of the epithelial lining of the respiratory tract. Grade III metaplasia.

Case 3.

0.487/54. Twenty-two-year-old teacher.

In 1951 protein was traced in the urine during the patient's military service. After a series of stays in hospital, the condition was interpreted as chronic nephritis which lasted until his death in October 1954. Urination, which had always inconvenienced him, was only possible by catheterization during the last days of his life. Blood urea 514 mg. per cent. At autopsy, considerable bilateral hydronephrosis and megalo-ureters were found with almost complete loss of kidney parenchyma. The condition was regarded as a sequel of congenital valve formation in the prostatic section of the urethra. No remains of normal kidney tissue were found microscopically. In the large and small bronchi, the epithelium was to a great extent flattened and stratified, as in Cases 1 and 2. Grade III metaplasia.

Case 4.

0.99/55. Eighteen-year-old schoolgirl.

Treated at home for anaemia about 3 months prior to her sudden death February 1955 without any exact diagnosis having been made. Autopsy revealed a very slim young woman. The lungs were relatively hyperaemic compared with the otherwise very pale organs. The kidneys were small, pale and granular with fibrosis and infiltration of lymphocytes and numerous hyalinized glomeruli (chronic glomerulo-nephritis). Blood urea post mortem was 556 mg. per cent

(possibly slightly lower during the last hours of life (Madsen, 1924)). The bronchial epithelium was partly squamous, especially in the upper lobe of the right lung (Fig. 3). Grade II metaplasia.

Case 5.

0.108/55. Thirty-five-year-old woman secretary.

In 1944 dysuria and protein in the urine with a progressive insufficiency of kidney function and rising blood pressure until her death in March 1955. Ten days before she died the blood urea level was 582 mg. per cent. Autopsy revealed huge polycystic kidneys (1110 g. and 1600 g.) with no remains of normal parenchyma. There were fibrous tuberculous changes in the periphery of the upper lobe of the right lung only. The bronchi in all sections of the lungs were covered to a considerable extent by a fairly regular 5–7 cell layer thick squamous epithelium (Fig. 4). The subepithelial connective tissue was hyperaemic and oedematous. Grade III metaplasia.

DISCUSSION

Squamous metaplasia of the bronchial epithelium is not uncommonly seen in routine autopsy material when thoroughly studied, but its presence seems to be rather prevalent in cases of uraemia. In each of 17 cases of chronic kidney disease with uraemia, metaplasia was found to have taken place.

None of these cases suffered from acute respiratory disease which according to the literature, may lead to epithelial metaplasia (Askanazy, 1919; Niskanen 1949; Wegelin, 1919; Weller, 1953.) and which was also present in several other non-uraemic cases with metaplasia.

As already mentioned, we cannot exclude the possibility that chronic irritants of various types (such as tobacco smoke and pollution of air by dust) to some extent may have affected these cases, particularly amongst the older patients. Neither can we, in spite of the negative information, completely ignore the possible influence of some chronic or acute respiratory infection.

It would appear that the role of irritants is of minor importance in the younger age group, and in none of 5 cases reported in detail did the previous history suggest any kind of irritation of the respiratory tract epithelium. In these cases, therefore, we must look for other explanations of the epithelial changes.

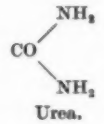
It is well known that considerable general, squamous epithelial metaplasia may develop in many organs, also extensively in the respiratory tract in cases of vitamin A deficiency (Sweet, 1935; Wolbach and Howe, 1925). It is possible to presume that kidney disease interferes in a decisive manner with the metabolism of vitamin A and thereby leads to metaplasia of the epithelium. The vitamin may be destroyed, or the absorption, transportation or storage interfered with, or the synthesis may be blocked in some way or other. Our investigations did not, however, elucidate these problems.

The uraemic condition in kidney failure, on the other hand, often leads to considerable pathological changes which may not have anything to do with the metabolism of vitamin A. Both uraemic pericarditis and uraemic colitis are well known conditions, the latter possibly due to vascular changes, which can often be demonstrated in the uraemic cases (Siegmund, Henke and Lubarsch, 1929), and which may lead to ulceration and necrosis of the intestinal mucosa. "The

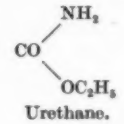
uraemic lung" is also a well known term, and changes may be found both by X-ray examination and morphologically in this condition (Bass and Singer, 1950; Ehrich and McIntosh, 1932). Microscopic examination of such lungs, reveals thickening of the alveolar septa, hyperaemia and fibrinous exudate. Bronchiolitis obliterans has been regarded as a possible last stage of such processes (Bass and Singer, 1950).

One reason why epithelial metaplasia has not been observed previously in these lungs may possibly be that autolysis of the bronchial epithelium takes place very rapidly if the material is not fixed at an early stage post-mortem. It is possible that the alteration of circulation, which to a great extent takes place in some uraemic cases, may lead to trophic disturbances of the mucosa with metaplasia as a result. The manner of differentiation of the respiratory tract epithelium may change, or perhaps ulceration occur, similar to that in the colon in uraemic colitis. Such ulcers will naturally heal with squamous epithelium which may be stratified in several layers and also irregular.

A direct influence on the epithelium from metabolic products in chronic kidney disease may also be taken into consideration. In this connection it may be mentioned that sarcomatous and adenomatous lung tumours have been produced by urethane injections in mice and rats. Urethane is chemically closely related to urea, which may be seen from the formulae:



Urea.



Urethane.

We cannot give any definite explanation of the cause of the epithelial metaplasia in our cases. The mechanism may equally well be interference with the vitamin A metabolism, chronic irritation and possible ulceration due to circulatory disturbance, or an "intrinsic" factor of metabolic origin due to the chronic kidney insufficiency. A combination of these factors may also come into consideration.

SUMMARY

1. In a comprehensive study of autopsy material, squamous epithelial metaplasia in the respiratory tract was observed remarkably often in individuals with uraemia, particularly in cases where the uraemia was a result of chronic or subacute kidney disease.

2. In the older patients some kind of chronic irritation, not related to the uraemia, may have played a role as the cause of the metaplasia, but in 5 young

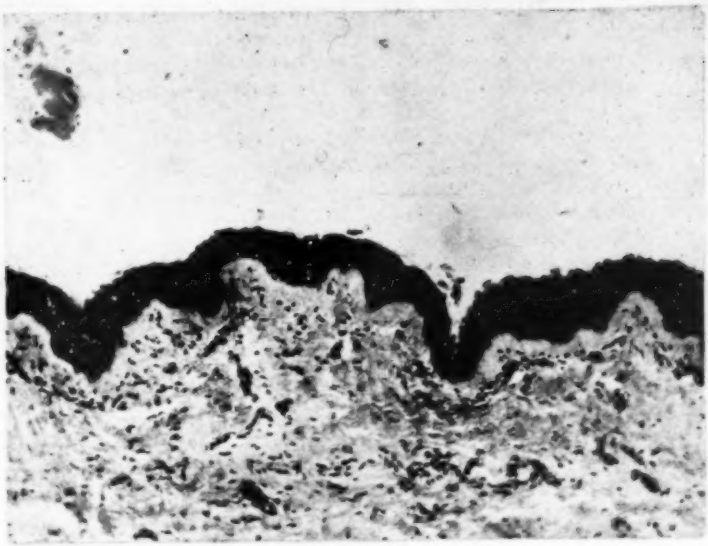
EXPLANATION OF PLATES

FIG. 1.—Case 1. Squamous epithelium of lower trachea. H. & E. × 115.

FIG. 2.—Same case. H. & E. × 700.

FIG. 3.—Case 4. Squamous epithelium of upper right lobe bronchus. Duct of submucous gland is blocked by epithelium. H. & E. × 210.

FIG. 4.—Case 5. Squamous epithelium of upper left lobe bronchus. H. & E. × 420.



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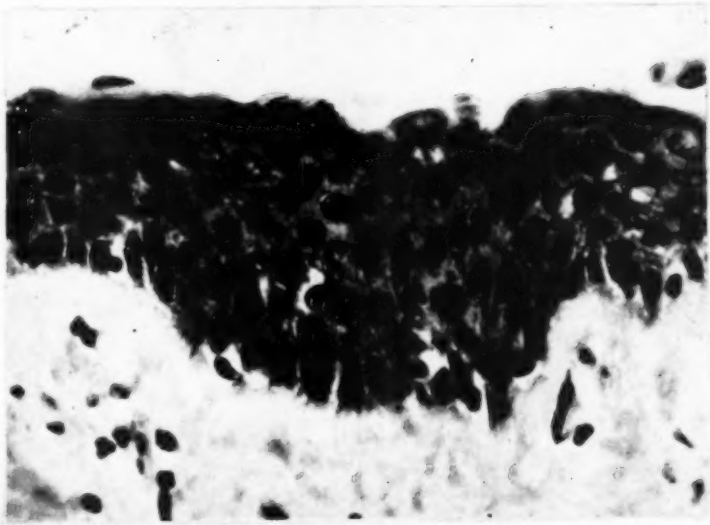

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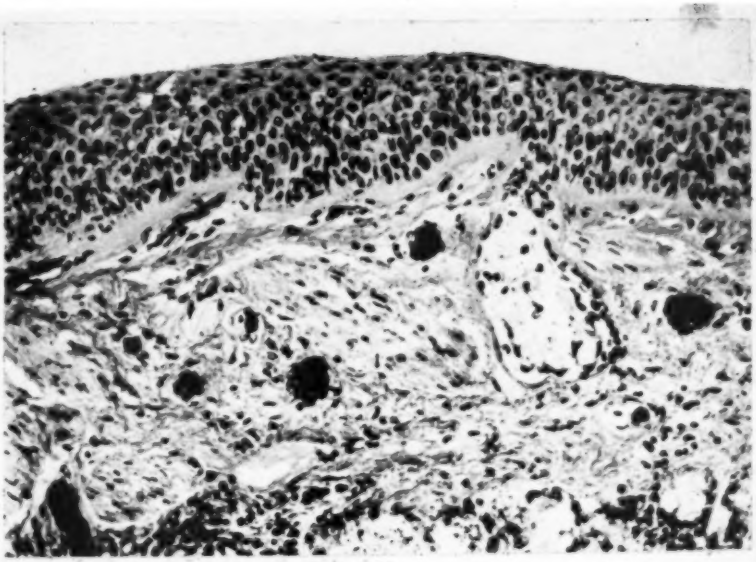

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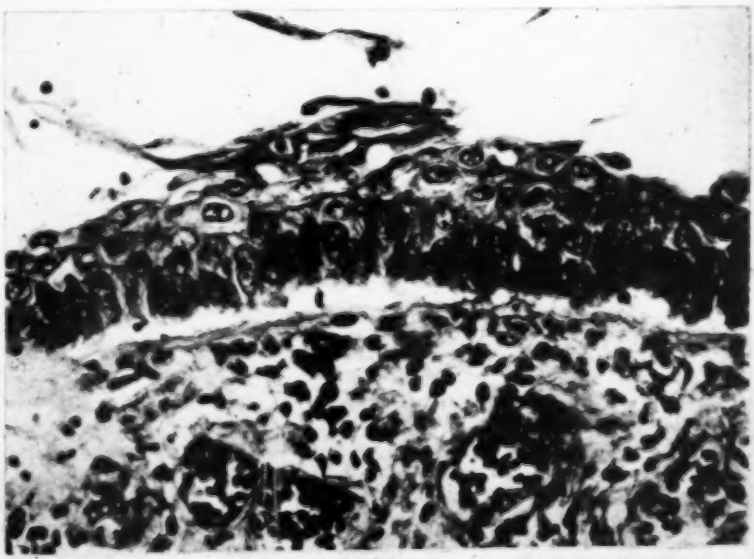

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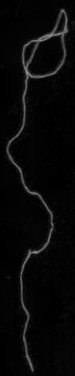

patients, chronic irritation could be ruled out. In these 5 cases with chronic disease of the kidneys, widespread metaplastic changes were found in the bronchi.

3. Possible causes of the epithelial changes in this condition are discussed.

REFERENCES

ASKANAZY, M.—(1919) *KorrespBl. schweiz. Ärz.*, **44**, 465.
PASS, H. E. AND SINGER, E.—(1950) *J. Amer. med. Ass.*, **144**, 819.
EHRICH, W. AND MCINTOSH, J. F.—(1932) *Arch. Path.*, **13**, 69.
MADSEN, St. Tschudi—(1924) Studier over restkvelstoffets og urinstoffets optraeden og fordeling specielt i den syke organisme. Bergen (J. W. Eide).
NISKANEN, K. O.—(1949) *Act. Path. Scand.*, Suppl. 80.
SEIGMUND, H., HENKE, F. AND LUBARSCH, O.—(1929) ' Handbuch der speziellen pathologischen Anatomie und Histologie,' IV/3 : 345, Berlin. (Julius Springer.)
SWEET, L. K. AND K'ANG, H. S.—(1935) *Amer. J. Dis. Child.*, **50**, 699.
WEGELIN, C.—(1919) *KorrespBl. schweiz. Ärz.*, **44**, 65.
WELLER, R. W.—(1953) *Amer. J. clin. Path.*, **23**, 768.
WOLBACH, S. B. AND HOWE, P. R.—(1925) *J. exp. Med.*, **42**, 753.

PRIMARY CARCINOMAS OF THE LIVER AND BILIARY TRACT

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Received for publication February 20, 1956.

THE classification of carcinomas arising in the liver into liver cell carcinoma and carcinoma of the intra-hepatic bile ducts was put forward by Goldzieher and von Bokay (1911) and supported by Yamigawa (1911). In the literature that has since appeared differences between these tumours in their behaviour, incidence and possible causative factors have not been sufficiently investigated or appreciated for them to be regarded as separate tumours. There has been a tendency to regard them as histological variants of a homogeneous group of tumours and to group them on a regional basis as carcinoma of the liver. Tumours of the liver have attracted special interest on account of their geographical and racial distribution.

This paper is based on 105 cases of carcinoma of the liver and biliary tract found among 9893 post-mortem examinations during a 6½ year period in Singapore, where, as in other parts of South-East Asia and South China, liver cell carcinoma occurs with alarming frequency. In presenting a summary of the pathological evidence gathered from this series, I shall endeavour to illustrate significant differences between carcinomas derived from the liver cells and bile ducts and to strengthen the view that the distribution of the disease is dependent on environmental rather than genetic factors.

The tumours belonged to the following groups:

Liver cell carcinoma (hepatocellular carcinoma)	86 cases
Carcinoma of the intra-hepatic bile ducts (cholangiocellular carcinoma)	8 cases
Carcinoma of the extra-hepatic bile ducts	5 cases
Carcinoma of the gall bladder	6 cases

Microscopic Features and Diagnostic Criteria

Liver cell carcinoma

The cytological appearance, the architectural pattern and the stromal reaction are usually distinctive. The tumour cells are usually polygonal and fairly large and have a resemblance to liver cells; the cytoplasm is abundant and granular and may occasionally contain droplets of bile or globules of fat. Cellular pleomorphism is frequently displayed and giant cells may dominate the picture. A trabecular structure consisting of anastomosing columns of cells reminiscent of the corded architecture of the normal liver is a striking microscopic feature which is nearly always evident if sufficient tissue is examined. The stroma is scanty and typically consists of little more than an endothelial lining investing the tumour cords and forming an "open" vascular network.

Structural variations are common: parts of the tumour may exhibit an alveolar pattern, there may be widespread areas of disorderly or "sarcomatous" structure and there may be a variable fibrous tissue stroma. Although individual characteristics preponderated in some tumours, they did not appear to represent

any fundamental difference or to provide a satisfactory basis either to subdivide the tumour into a number of histological types or to grade them according to degree of malignancy.

The exhibition of an alveolar pattern by liver cell carcinoma has been widely accepted (Stewart, 1931 ; Ewing, 1940 ; Berman, 1951 ; Edmondson and Steiner, 1954), but although Tull (1932) is perhaps the only author to deny this the histologic discriptions in literature suggest that carcinomas of the liver have sometimes been classified as cholangiocellular carcinoma because a variable portion had shown an alveolar structure. It is therefore emphasised that an alveolar structure occurs frequently in liver cell carcinoma. It was found in 29 cases in this series : cleft-like spaces were seen in many tumour cords and these could be traced into well developed lumina ; some tumours consisted largely of alveoli lined by single layers of cells.

Carcinomas of the biliary tract

Carcinoma of the intra-hepatic ducts.—These tumours are usually schirrous adenocarcinomas but may display papillary, mucoid or anaplastic features. The cells are cubical or columnar, resemble biliary epithelium and lack the granularity and occasional eosinophilic character shown by liver cell tumours. Bile formation is not exhibited but necrotic tumours may show some biliary impregnation from without. All the tumours in this series presented an alveolar appearance ; there was evidence of mucus secretion in three cases and in one there was squamous metaplasia. The necropsy findings in the case with squamous metaplasia were those of a large primary hepatic tumour with involvement of the extra-hepatic portion of the right hepatic duct, and numerous liver flukes (*Clonorchis sinensis*) were found in the intra-hepatic ducts. Metastases were found in the lymph nodes at the porta hepatis, the myocardium of the left ventricle and the subpleural tissues of both lungs ; the respiratory passages and mediastinal nodes were normal. Microscopically, the hepatic tumour and all the metastases showed an adenocarcinomatous structure intimately mixed with areas of squamous-celled structure with " nest " formation and areas of transitional appearance. This microscopic variant of cholangiocellular carcinoma has not been previously described but Mallory (1930), reporting a case of squamous cell carcinoma where an enormous tumour of the liver was associated with tumour involvement of the upper quarter of the common bile duct and retro-peritoneal lumph nodes, made a diagnosis of carcinoma of the extra-hepatic duct because this was the only organ among those involved where a squamous carcinoma was known to occur.

Carcinoma of the extra-hepatic ducts and gall bladder.—The majority of these tumours are infiltrating adenocarcinomas with schirrous, mucus-producing and papillary varieties ; anaplastic and squamous varieties are less common. In this series, three of the carcinomas of the extra-hepatic ducts were schirrous adenocarcinomas with mucoid changes in two of them, one was a diffusely infiltrating signet-ring cell carcinoma and one an anasplastic spheroidal cell carcinoma. Among the gall bladder carcinomas there were three columnar cell adenocarcinomas with mucoid changes, two schirrous adenocarcinomas and one anaplastic carcinoma.

There is no essential histologic difference between carcinomas arising from different parts of the biliary tract. These tumours are not distinguishable microscopically but it has been observed in studies of large series of cases that dense fibrosis is more common in carcinomas of the extra-hepatic ducts and that mucoid,

papillary and metaplastic features are more commonly associated with those of the gall bladder.

Hepatic tumours with intermediate or mixed features

Cases of hepatocellular and cholangiocellular carcinoma may rarely be found where the typical sharply contrasting microscopic features are not evident and are replaced by intermediate or mixed features. Such tumours have been described by several writers (Wells, 1903; Koster and Kasman, 1932; Bonne, 1937; Gustafson, 1937; Mallory, 1940; Ewing, 1940; Allen and Lisa, 1949; Liu, 1953; Edmondson and Steiner, 1954) who have variously designated them "mixed tumour", "duplex type", "intermediate type", "carcinoma of dual origin", "cholangiohepatoma", "hepatobiliary carcinoma" and "combined liver cell and bile duct carcinoma". The recorded cases have either been separate neoplastic masses which occasionally fused in collision or individual mases displaying an intimate mixture of both features. The term "cholangiohepatoma" and its synonyms imply a single neoplasm that is the product of a simultaneous malignant change in the liver cells and bile ducts. It is doubtful if such tumours ever occur and the fact that most of the recorded tumours have been in extensively cirrhotic livers (Ewing, 1940) and in males suggests that many of them are histological variants of liver cell carcinoma; such a conclusion has indeed been reached by some authors (Liu, 1953; Edmondson and Steiner, 1954). Should separate hepatocellular and cholangiocellular tumours occur fortuitously in the same liver it would be preferable to name them individually and to avoid the above-mentioned terms even if they have fused in places. Single tumours with mixed features would probably reveal their identity if more tissue is examined microscopically, and in the exceptional instance where the diagnosis remains in doubt it would be preferable to classify the tumour as a hepatic carcinoma of undetermined origin.

Gross Pathology

Liver cell carcinomas (86 cases)

The majority of tumours appear as multiple nodules or as massive growths measuring from 10–20 cm. in diameter with a few outlying nodules; less often they appear as poorly defined infiltrations. They are usually soft or pulpy, become crumbling and pultaceous with the onset of massive necrosis and present a variegated appearance due to haemorrhage and biliary impregnation. The hepatic surface commonly presents bosses of tumour tissue which in some instances may even be larger than the rest of the liver and project down to the pelvic brim. Rupture of the tumour with intra-abdominal haemorrhage is a frequent termination (19 cases in this series); in exceptional cases this may be the first clinical manifestation of the disease. With few exceptions the liver is appreciably enlarged; in this series the weights of the liver ranged from 870 g. to 6805 g. with an average weight of 2730 g.

The site of origin: The tumours are usually widely disseminated throughout the liver and it is often difficult to decide on the primary focus of origin. In his monograph on hepatic carcinoma, Berman (1951) recorded a remarkably high tumour incidence in the right lobe and stated that it was highly probable that carcinogenic agents absorbed from the small intestine may be "streamlined"

through the portal vein into the right lobe of the liver. The findings in the present series which are set out below do not support this view :

Tumours involving right lobe more extensively	39
Tumours involving left lobe more extensively	12
Tumours distributed evenly throughout the organ	35
Tumours restricted to the right lobe	15
Tumours restricted to the left lobe	3

The distribution of the tumour in the liver approximates roughly with the relative bulk of the two lobes and does not indicate that the cells in the right lobe are especially prone to develop carcinoma. A comparison of tumours restricted to each lobe shows a relatively greater disparity as the much larger size of the right lobe permits a tumour to be confined to it even after considerable growth.

Changes in the liver : Portal cirrhosis of varying severity was found in 81 (94 per cent) cases in this series ; two of them were cases of hepatic schistosomiasis and one of haemochromotosis. There were no significant abnormalities in the biliary tract.

Growth and metastases : Direct infiltration of the liver was seen in all the cases in this series and in 10 cases there was invasion of the adherent diaphragm. Gross tumour extensions into branches of the portal vein and, less frequently, the hepatic veins were present in 70 (81 per cent) cases and constituted one of the most striking macroscopic features. The main trunk of the portal vein was often distended with tumour tissue and blood clot ; in 6 cases the tumour extended into the inferior vena cava and in three of these it extended further into the cavity of the right atrium. There were frequent and widespread intra-hepatic metastases but blood-borne extra-hepatic metastases were found in only 28 (32 per cent) cases (lungs in 25, stomach in 3, pancreas in 1). Lymph nodal metastases restricted to the abdomen were found in 12 (14 per cent) cases and peritoneal metastases in 2 cases.

The relative infrequency of extra-hepatic metastases in hepatocellular carcinoma is of interest in view of the extreme venous invasion that is often demonstrable and the readiness with which carcinomas of the biliary tract metastasize. In considering this difference, which has been reported previously (Ewing, 1940 ; Hoyne and Kernohan, 1947 ; Edmondson and Steiner, 1954) and is probably due to an inherent character of the tumour cells and their environmental requirements, it may be pertinent to quote the experimental results of Cameron and Oakley (1934) who observed an analogous difference between liver cells and duct cells. In a study of autoplastic transplants of the liver in rats they found that whereas the bile ducts successfully maintained their existence for a long time the liver cells soon began to degenerate and finally dissappeared.

Clinical features : The main symptoms are abdominal pain, abdominal swelling and asthenia and the main physical signs are emaciation, hepatomegaly, ascites, engorged abdominal veins and jaundice. A clinical diagnosis was made in most cases in this series but it was sometimes obscured by the absence of hepatomegaly and when the picture was overshadowed by such complications as abdominal haemorrhage, haematemesis, hepatic coma and intercurrent illnesses. Metastatic symptoms and pyrexia have predominated in some recorded cases. The disease runs an extremely rapid course ; this is due mainly to rapid tumour growth and partly to the " reserve " of the liver which permits a tumour to remain latent until it has grown considerably. Most of the cases in this series (92 per cent) died

within 6 months of the onset of symptoms. The following causes of death were established at necropsy :

Cachexia with varying degrees of liver failure and venous obstruction	50 cases
Rupture of tumour with abdominal haemorrhage	19 „
Rupture of oesophageal varices with haemorrhage	5 „
Tumour invasion of inferior vena cava and extension into right atrium	3 „
Pulmonary artery occlusion by tumour emboli	1 „
Terminal pneumonia	2 „
Other diseases	3 „
Suicide	3 „

Carcinomas of the biliary tract (19 cases)

Carcinoma of the intra-hepatic ducts.—These tumours may also assume the form of massive, multinodular or diffusely infiltrating growths but are characteristically firm and homogeneous and do not exhibit the soft texture, extensive necrosis and variegated appearance of hepatocellular carcinoma (Snijders and Straub, 1923 ; MacCallum, 1928 ; Ewing, 1940). They show a greater tendency to involve the hilar portion of the liver and the gross appearances may suggest duct origin : in one of the cases in this series there was localised hydrohepatosis following malignant obstruction of an intra-hepatic duct, in another there was polypoidal tumour extension into the duct lumen and in a third there were warty tumour nodules in the wall of a bile duct cyst associated with tumour nodules in the liver. Five of the tumours involved the right lobe more extensively and three the left.

Changes in the liver : Portal cirrhosis was demonstrated in only one case. The intra-hepatic ducts contained calculi in 3 cases and liver flukes (*C. sinensis*) in one case.

Growth and metastases : The massive venous invasion characteristic of hepatocellular carcinoma is not found but metastases are relatively frequent and widespread. In this series there were blood-borne metastases in five of the eight cases (lungs 5, pancreas 1, kidney 2, heart 2, bones 3, dura mater 2). Spread along the lymphatics and bile ducts may lead to involvement of the extra-hepatic ducts ; this was found in 3 cases.

The clinical features resemble those of hepatocellular carcinoma but it may be observed that the symptoms of abdominal haemorrhage and extreme venous invasion are not features of cholangiocellular carcinoma. They are as rapidly fatal as liver cell tumours ; all the cases in this series died within 6 months of onset of symptoms, death being attributed to cachexia (3 cases), metastases (2 cases), terminal pneumonia (1 case) and other diseases (2 cases).

The macroscopic appearances and behaviour of cholangiocellular carcinoma were found to resemble those of carcinoma of the extra-hepatic biliary tract which are usually diffusely infiltrating schirrous tumours and may exhibit polypoid or mucoid characteristics. The hepatic changes depend on the situation and duration of the tumour and jaundice is frequently the major symptom. Direct invasion of the liver and widespread blood-borne metastases are frequently found.

Incidence and Causative Factors

Geography and race

Berman (1951) presented a detailed comparison of the incidence of hepatic carcinoma in various countries and races based on necropsy records. Although

variations in the composition of different necropsy series render such a comparison unreliable necropsy figures do provide an approximate guide and uphold the generalisation that carcinoma of the liver is more frequent among the peoples of Asia and Africa than in those of Europe and America.

The composition of the present necropsy series and the distribution of the tumours are shown in Tables I and II. Liver cell carcinoma was present in 0·88

TABLE I.—*Age, Race, and Sex Distribution of this Necropsy Series*

Age.	Chinese.		Indians and Pakistanis.		Malaysians.		Others.		Total.	
(years.)	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
0–9	2172	1657	66	52	32	25	18	5	2288	1739
10–19	355	167	27	19	32	7	7	1	421	194
20–29	496	203	106	23	80	12	36	8	718	246
30–39	781	251	184	30	48	9	31	6	1044	296
40–49	910	228	157	10	34	8	30	8	1131	254
50–59	733	121	106	8	23	7	34	3	896	139
60–69	308	57	24	7	3	2	9	3	344	69
Over 70	67	32	3	1	3	2	5	1	78	36
Total	5822	2716	673	150	255	72	170	35	6920	2973
	8538 (86·3%)		823 (8·32%)		327 (3·31%)		205 (2·07%)		9893	

TABLE II.—*Distribution of Tumours*

	Male.	Female.	Total.	Chinese.	Indians and Pakistanis.	Others.
Liver cell carcinoma	81	5	86	82	4	—
Carcinoma of the intrahepatic bile ducts	8	—	8	7	1	—
Carcinoma of the extra-hepatic bile ducts	5	—	5	5	—	—
Carcinoma of the gall bladder	6	—	6	5	1	—
Total carcinomas	348	78	426	394	22	10
Total malignant tumours	416	112	528	478	31	19
Total necropsies	6920	2973	9893	8538	823	532

per cent of all necropsies, carcinoma of the intra-hepatic ducts in 0·08 per cent, carcinoma of extra-hepatic ducts in 0·05 per cent and carcinoma of the gall bladder in 0·06 per cent. These percentages are low on account of the selected nature of the necropsies: a large proportion (41 per cent) was on children below 10 years and approximately half the total necropsies were on Coroners' cases. Liver cell carcinoma was the most frequent malignant tumour among necropsies and constituted 20 per cent of all carcinomas; records of biopsy examinations, however, show that carcinomas of the cervix uteri and nasopharynx are both more frequent in Singapore.

The frequency of hepatic carcinoma among Oriental and African peoples appears to be largely due to the high frequency of liver cell carcinoma; it cannot be shown that cholangiocellular carconoma occurs more frequently among these

peoples. In the U.S.A. the following ratios of hepatocellular to cholangiocellular carcinomas have been recorded : Smith (1933)—19 : 6 (3·2 : 1) ; Gustafson (1937)—39 : 21 (1·9 : 1) ; Wilbur, Wood and Willet (1944)—45 : 4 (11·3 : 1) ; Hoyne and Kernohan (1947)—20 : 11 (1·8 : 1) ; Edmondson and Steiner (1954)—75 : 25 (3·0 : 1); Overton, Kaden and Livesay (1955)—49 : 12 (4·1 : 1). In contrast the recorded ratios among the Bantus have been 28 : 3 (9·3 : 1) in the series of Pirie (1921) and 56 : 4 (14·0 : 1) in that of Berman (1951). Similarly, Snijders and Straub (1923) found a ratio of 79 : 4 (19·8 : 1) among the Chinese and Javanese in Sumatra, and in the present series the ratio was 82 : 7 (11·7 : 1) among the Chinese. Tull (1932) recorded a ratio of 99 : 35 (2·8 : 1) in a series from Singapore but this high proportion of cholangiocellular carcinoma is almost certainly due to his non-acceptance of any tendency of liver cell carcinoma to assume an alveolar pattern.

The predisposition of certain races to develop hepatic carcinoma raises the question of whether it is due to genetic factors or to environmental factors or to both. The high incidence of hepatic carcinoma in certain strains of laboratory mice bred from an ancestry with hepatic tumours (Slye, 1916 ; Gorer, 1940 ; Pybus and Miller, 1942) has lent support to the theory that the tendency to develop the disease is inheritable. There has, however, been no real evidence of such an inherited tendency in humans in whom the controlling influence of environmental conditions is shown by the relatively low incidence of hepatic carcinoma in the

EXPLANATION OF PLATES

Liver cell carcinoma

FIG. 1.—Portion of tumour showing lobular structure closely resembling that seen in portal cirrhosis, but with no interlobular ducts. × 20.
FIG. 2.—Typical arrangement of tumour cells in anastomosing cords. × 85.
FIG. 3.—Thick tumour columns separated by endothelium-lined spaces. × 85.
FIG. 4.—Canalisation of tumour columns. × 85.
FIG. 5.—Deeply stained plugs of bile in several tumour alveoli. × 85.
FIG. 6.—Tumour cells showing some cytological resemblance to normal liver cells. × 340.
FIG. 7.—Cellular pleomorphism in anaplastic tumour ; several multinucleated tumour cells. × 340.

Carcinoma of the intra-hepatic bile ducts

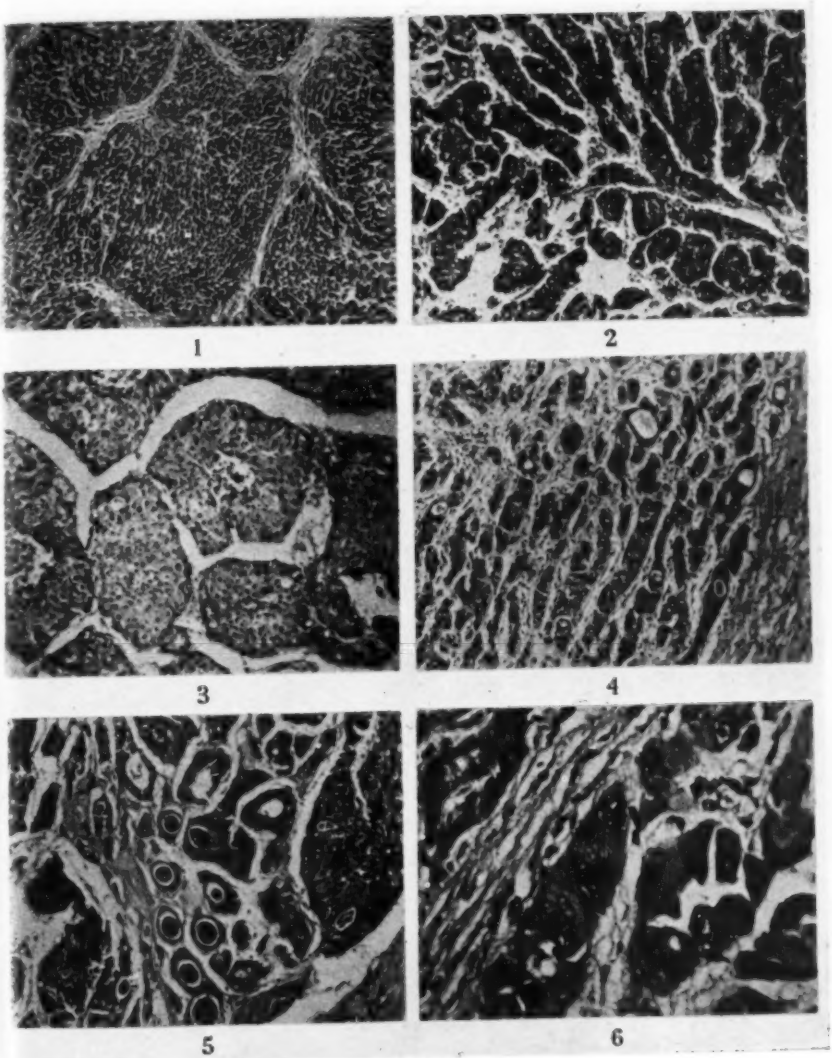
FIG. 8.—Tumour showing adenocarcinomatous structure with some adjoining normal liver tissue. × 85.
FIG. 9.—Tumour alveoli separated by dense fibrous stroma. × 85.
FIG. 10.—Cytological appearance of tumour shown in Fig. 9. × 340.
FIG. 11.—Tumour showing metaplastic squamous cell features in addition to adenocarcinomatous structure. × 85.
FIG. 12.—Another portion of tumour shown in Fig. 11 exhibiting a predominantly squamous structure ; a zone of surviving liver tissue is seen in the middle. × 85.

Liver cell carcinoma

FIG. 13.—" Massive " variety of liver cell carcinoma.
FIG. 14.—" Multinodular " variety of liver cell carcinoma ; extensive filling of branches of portal vein with tumour tissue.
FIG. 15.—" Diffusely infiltrating " variety of liver cell carcinoma.

Carcinoma of the intra-hepatic bile ducts

FIG. 16.—Warty tumour nodules in wall of bile duct cyst associated with conglomerate tumour nodules in one half of liver ; a small white probe has been placed in duct leading from cyst.
FIG. 17.—Tumour of right lobe of liver extending into lumen of right hepatic duct.



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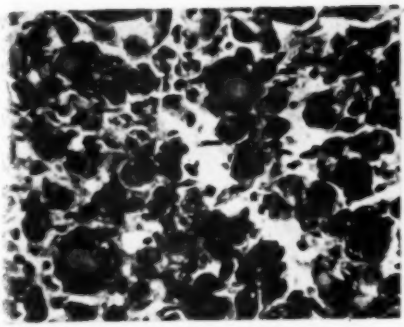

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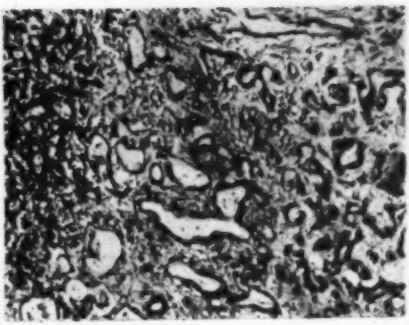

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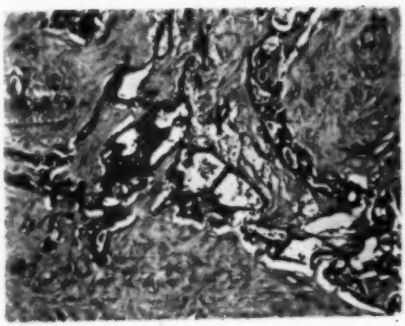

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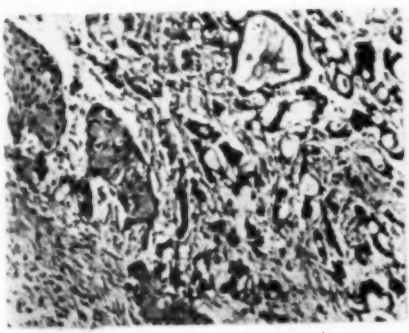

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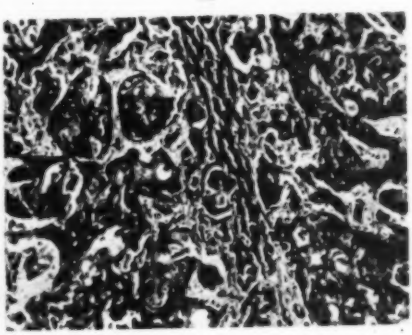

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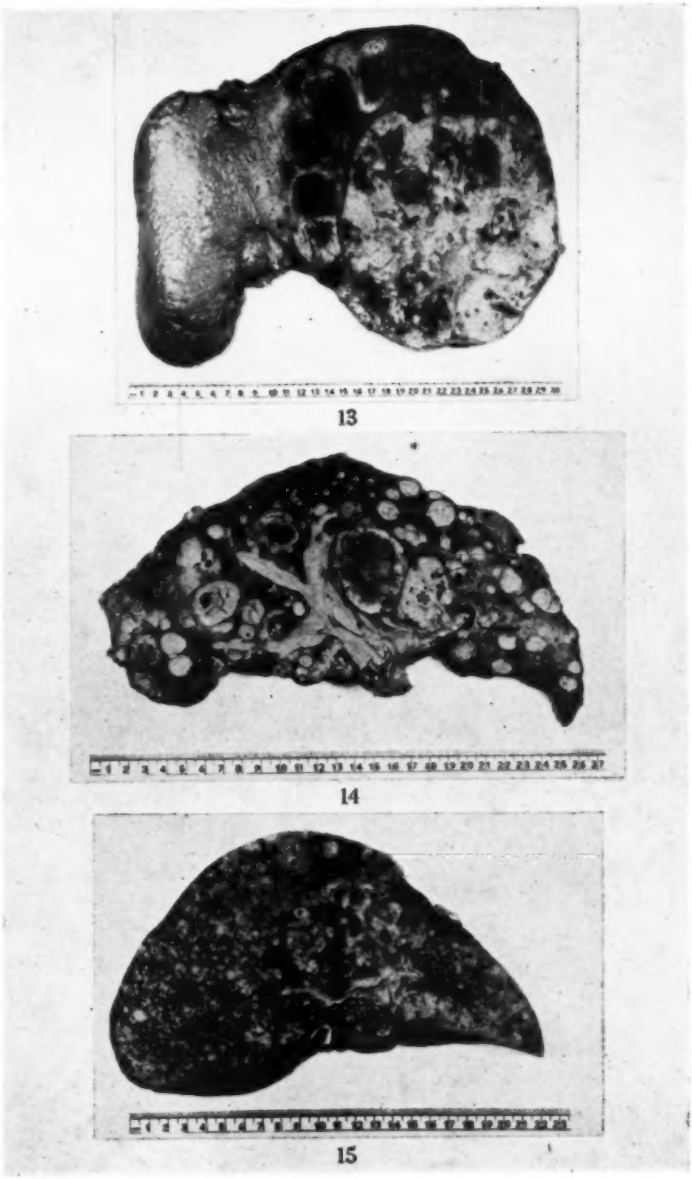

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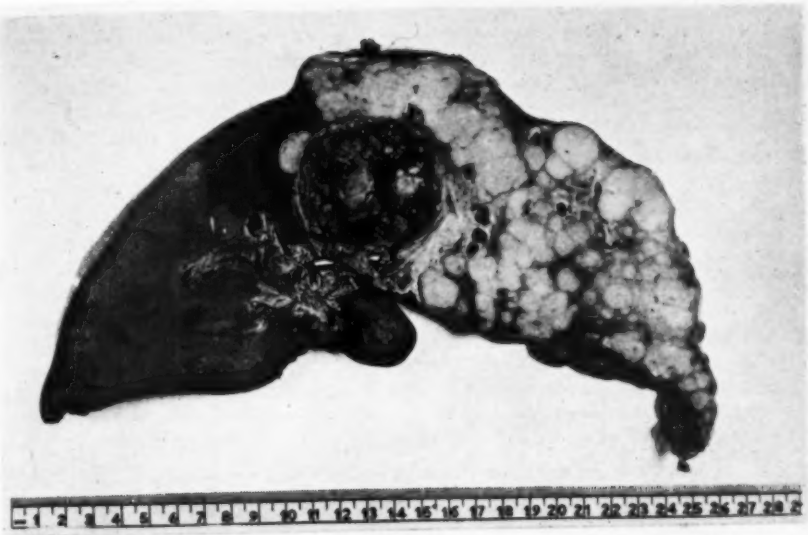

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American Negro (Kennaway, 1944) and the distribution of the disease in Africa (Gillman, Gillman and Gilbert, 1950).

Chinese patients have figured prominently in series of hepatic carcinoma in Western countries but most of them have been China-born immigrants who have been in their new environment for relatively short periods. The high incidence among Chinese in this series was restricted to the immigrants and those born in Singapore showed no undue susceptibility to the disease (Table III). Of the 73 cases of liver cell carcinoma where information regarding the place of birth was available only one was born in Singapore, four were born in neighbouring countries and the remaining 68 cases (93 per cent) occurred among those born in China, all of whom were from the southern provinces of Kwangtung and Fukien and the island of Hainan off the South China coast. An overwhelming majority of the Chinese immigrants in Singapore are also from these parts of South China. All four Indians with liver cell carcinoma were born in India.

A relatively greater number of Singapore-born Chinese belong to the lower age groups and, as 95 per cent of liver cell carcinomas occurred above the age of 30 years, the relatively high incidence among China-born Chinese in Table III is also due to selection. The age distribution of the census figures is not available but that of 1000 consecutive Chinese patients is provided in Table IV for compari-

TABLE III.—*An Analysis of the Place of Birth of Cases of Carcinoma in Chinese where such information was available, of* 1000 *consecutive Chinese Patients and the Chinese Population of Singapore*

	Total.	Number born in Singapore.	Number born in China.	Number born elsewhere.
Chinese population in Singapore (1947 census)	73,013	421,406 (57·72%)	281,032 (38·49%)	27,695 (3·79%)
Consecutive admissions to medical wards	1,000	541	438	21
Liver cell carcinoma	73	1	68*	4
Carcinoma of the intra-hepatic bile ducts	7	—	7	—
Carcinoma of the extra-hepatic bile ducts	5	—	5	—
Carcinoma of the gall bladder	3	—	3	—

* The period since arrival in Singapore was stated to be less than 10 years in 8 cases, 10–19 years in 15 cases, 20–29 years in 14 cases, 30–39 years in 14 cases, 40–49 years in 4 cases and was unknown in 13 cases.

TABLE IV.—*The Age and Place of Birth of the* 1000 *Consecutive Chinese Patients Referred to in Table III*

Age.	Number of patients born in Singapore.	Number of patients born in China.	Average period since arrival in Singapore.	Number of patients born elsewhere.
0–9	64 (98·5%)	0	—	1
10–19	179 (95·2%)	6	6·3 yrs.	3
20–29	129 (73·7%)	44	11·4 yrs.	2
30–39	81 (50·0%)	71	12·8 yrs.	10
40–49	45 (27·3%)	116	14·3 yrs.	4
50–59	26 (17·7%)	121	22·4 yrs.	—
60–69	15 (18·8%)	64	24·4 yrs.	1
70 and over.	2 (11·1%)	16	28·8 yrs.	—
Total	541 (54·1%)	438	—	21

son. The ratio of China-born to Singapore-born Chinese was 2·3 : 1 among patients over 30 years but there were 68 liver cell carcinomas and 15 carcinomas of the biliary tract among China-born Chinese and only one liver cell carcinoma occurred in a Singapore-born Chinese. These figures indicate that the difference between the two groups, although exaggerated in Table III, is nevertheless significant. As there is hardly any genetic difference between the immigrant Chinese and the indigenous Chinese of Singapore of whom a great majority have been in Singapore for less than 3 to 4 generations, these findings indicate that the occurrence of the tumour was not due to any inherited susceptibility but to some difference in the environment or mode of life to which these groups of people have been subjected.

Age

The cases of liver cell carcinoma ranged from 19 years to 74 years with a maximum frequency in the fifth decade (43 per cent of cases) followed by the sixth decade (30 per cent of cases) and the fourth decade (14 per cent of cases). A liver cell carcinoma found in an infant aged 13 months was considered to be probably a hepatoblastoma and excluded from this series.

The age distribution of carcinoma of the intra-hepatic ducts was between 40 and 70 years, that of carcinoma of the extra-hepatic ducts between 37 and 60 years and that of carcinoma of the gall bladder between 45 and 68 years. Ewing (1940) observed that cholangiocellular carcinoma tends to occur at later ages than liver cell carcinoma.

Sex

An overwhelming majority of liver cell carcinomas occurs in males. In this series males constituted 93 per cent of all cases of liver cell carcinoma but only 70 per cent of all necropsies. The tumour was found in 1·2 per cent of necropsies on males and 0·2 per cent of necropsies on females. It is evident from these and all other published figures that the marked male predominance is due to a true sex predilection in addition to the preponderance of males in most necropsy series. Furthermore, it has been observed that male animals are more prone to develop experimentally produced liver cell carcinoma.

On the other hand, accounts in the literature indicate that females are more frequently affected by carcinomas of the biliary tract. In this series, however, all the carcinomas of the biliary tract occurred in males, but in view of the small numbers of these tumours and the fact that more than 80 per cent of necropsies above the age of 30 years were on males, no conclusion is drawn regarding sex predisposition. The decided frequency of carcinoma of the gall bladder among females is well known (Mohardt, 1939 ; Arminsky, 1949). Carcinoma of the extra-hepatic ducts has been recorded more frequently in males but published figures show that the sexes are almost equally susceptible (Kirshbaum and Kozoll, 1941 ; Neibling, Dockerty and Waugh, 1949 ; Willis, 1953). An analysis of recorded cases indicates that carcinoma of the intra-hepatic ducts occurs more frequently in females (Gustafson, 1937 ; Hoyne and Kernohan, 1947 ; Charache, 1939 ; Ewing, 1940 ; Edmondson and Steiner, 1954).

Portal cirrhosis

Portal cirrhosis is the most frequent precancerous lesion associated with liver cell carcinoma ; it is not commonly associated with carcinomas of the biliary

tract. In this series it was present in 81 (94 per cent) cases of liver cell carcinoma, one case of carcinoma of the intra-hepatic ducts, one of carcinoma of the extra-hepatic ducts and none of carcinoma of the gall bladder. The striking difference between the incidence of cirrhosis in hepatocellular and cholangiocellular carcinomas has been acknowledged by many writers (Goldzieher and Bokay, 1911; Smith, 1933; Stewart, 1931; Snijders and Straub, 1923; Liu, 1953; Willis, 1953; Gustafson, 1937; Greene, 1939; Edmondson and Steiner, 1954).

The frequency of carcinomatous change in cirrhotic livers was found to be 3·4 per cent by Stewart (1931) in a series from England and 9·3 per cent by Rosenthal (1932) in a series from America; in an analysis of 1989 cases of cirrhosis reported by different authors in Europe and America, Berk and Lieber (1941) found carcinomatous change in 4·5 per cent. Liu (1953) observed malignant transformation in 12·6 per cent of cases of cirrhosis in a series from China and Kouwenaar (1932) estimated that it occurred in 20 to 25 per cent of cirrhotic livers among the Javanese.

From the findings in this series, shown in Table V, several observations may be made. Firstly, cirrhosis is shown to be relatively prevalent, being demonstrable

TABLE V.—*The Incidence of Cirrhosis and the Frequency of Associated Liver Cell Carcinoma in this Necropsy Series*

	Frequency of cirrhosis in total necropsies.	Frequency of cirrhosis in necropsies over 30 years.	Frequency of liver cell carcinoma in cases of cirrhosis.
Chinese			
Males	257 (4·4%)	240 (8·6%)	73 (28·4%)
Females	44 (1·6%)	38 (5·5%)	5 (11·4%)
Total	301 (3·5%)	278 (8·0%)	78 (25·9%)
Indians and Pakistanis			
Males	31 (4·6%)	26 (5·5%)	3 (9·7%)
Females	3 (2·0%)	3 (5·4%)	—
Total	34 (4·1%)	29 (5·5%)	3 (8·8%)
Malaysians			
Males	9 (3·5%)	9 (8·1%)	—
Females	1 (1·4%)	1 (3·6%)	—
Total	10 (3·1%)	10 (7·2%)	—
Others			
Males	7 (4·1%)	7 (6·4%)	—
Females	1 (2·9%)	—	—
Total	8 (3·9%)	7 (5·4%)	—
Total			
Males	304 (4·4%)	282 (8·1%)	76 (25·0%)
Females	49 (1·7%)	42 (5·3%)	5 (10·2%)
Total	353 (3·6%)	324 (7·6%)	81 (23·0%)

in 3·6 per cent of all necropsies and 7·6 per cent of necropsies over the age of 30 years. Secondly, there was an unusually high rate of malignant transformation in cirrhotic livers especially among the Chinese; 23 per cent of all cases of cirrhosis showed liver cell carcinoma. Thirdly, malignant transformation in cases of cirrhosis was decidely more frequent in males (25 per cent) than females (10·2 per cent). Edmondson and Steiner (1954) have also observed that women with cirrhosis are less likely to develop liver cell carcinoma.

Infective hepatitis

Sheldon and James (1948) and Walshe and Wolff (1952) have recorded cases of liver cell carcinoma following hepatitis. Hepatitis is a fairly common disease in Singapore but there is no accurate information regarding its incidence.

Haemochromatosis

The association of liver cell carcinoma with haemochromatosis is well known, and although some authors have held the view that this is due to the coexistent cirrhosis (Stewart, 1922 ; Rosenthal, 1932 ; Sheldon, 1935), the consensus of opinion is that it is more frequent than in uncomplicated cirrhosis (Rowen and Mallory, 1925 ; Althausen and Kerr, 1927 ; Gustafson, 1937 ; Berk and Lieber, 1941). The only case of haemochromatosis in this series was associated with liver cell carcinoma.

Carcinoma of the intra-hepatic ducts, on the other hand, is not associated with haemochromatosis ; the only case on record is by Oshlag, Martin and Binford (1945) but their histologic description that the tumour consisted of cords and acini of large polyhedral cells arranged in a lobular pattern somewhat like that of pre-existing liver is typical of liver cell carcinoma.

Dietary factors

The distribution of hepatic carcinoma among peoples in whom malnutrition is common, and the observation that hepatic disease may follow dietary deficiencies and the oral administration of toxic substances have led to the supposition that the main causal factor in the production of liver cell carcinoma is dietary. This view is strengthened by Copeland and Salmon's (1946) observation that deficient diets may predispose to hepatic carcinoma in experimental animals. Such a relationship may well play a part in man but has not been proved.

Cholelithiasis

There is a close association of gall stones with carcinoma of the gall bladder (Mohardt, 1939 ; Arminsky, 1949) and, to a lesser extent,with carcinoma of the extra-hepatic ducts (Marshall, 1932 ; Ewing, 1940 ; Kirshbaum and Kozoll, 1941 ; Neibling, Dockerty and Waugh, 1949) ; in both instances the occurrence of calculi is much greater than in any control group of cases and is probably related to the development of carcinoma.

Cholelithiasis is exceptionally rare in liver cell carcinoma but is more frequently associated with carcinoma of the intra-heaptic ducts. Sanes and MacCallum (1942) reported two cases of cholangiocellular carcinoma associated with hepatolithiasis and put forward the view that a calculous cholangitis was a possible causative factor ; Brunschwig's (1953) case of a hepatic carcinoma in a woman who had an operation for calculi was also stated to be a cholangiocellular carcinoma. In this series gall stones were present in the gall bladder in only 2 (2·3 per cent) cases of liver cell carcinoma but were present in 3 of the 8 cases of carcinoma of the intra-hepatic ducts, in two of which they were located within the liver in association with the primary growth.

Parasites

A causal relationship between hepatic carcinoma and parasitic infestations of the liver has been supported in view of the high frequency of schistosomiasis in

South Africa (Pirie, 1921) and clonorchiasis in South China (Maxwell, 1928; Strong and Pitts, 1930 and 1932; Liu, 1953). It is not unreasonable to suppose that parasites may predispose to tumour growth but the existing evidence in favour of such a relationship is insufficient and consists only of a certain similarity in the geographical distribution of both diseases and their occasional coexistence in the same case. In Java and Sumatra, where schistosomiasis is not indigenous, helminthic infestations have played no part in the incidence of hepatic carcinoma (Bonne, 1935; Hartz, 1945). Hoeppli (1933) studied 66 cases of clonorchiasis and found only one case with portal cirrhosis and none with either carcinoma or any change that could be regarded as a precancerous stage. In a study of 500 cases of clonorchiasis, Hou (1955) found no direct relationship between parasitic infestation and portal cirrhosis but observed that a malignant change in the bile ducts was one of the most serious consequences of the disease.

Clonorchiasis and schistosomiasis are unknown among the indigenous people of Singapore who have not been shown to be prone to develop hepatic carcinoma. Among the immigrant Chinese in this series there were 8 cases of *S. japonicum* infestation of the liver in two of which there was associated liver cell carcinoma, and 67 cases of *C. sinensis* infestation among which there was one case of cholangiocellular carcinoma and none of liver cell carcinoma.

Discussion

An analysis of the findings in this series together with data culled from literature shows that there are several significant differences between liver cell carcinoma and carcinoma of the intra-hepatic bile ducts besides their microscopic features. Liver cell carcinoma occurs predominantly in males and especially among certain Oriental and African peoples. Portal cirrhosis is found in a majority of cases and appears to be the most significant amoug the various predisposing factors that have been suggested; further investigation is necessary before the roles of dietary deficiencies, infective hepatitis and parasitic infestations may be assessed. The growth of the tumour is characteristic by extensive venous invasion but extra-hepatic metastases are surprisingly infrequent. On the other hand, carcinoma of the intra-hepatic bile ducts occurs more frequently among females and does not show the racial predilection or the association with portal cirrhosis observed in liver cell carcinoma. It appears possible that the presence of parasites or calculi in the intra-hepatic ducts may be predisposing factors. Furthermore, extra-hepatic metastases are frequent and widespread although gross tumour invasion is not featured.

In all the aforementioned points carcinoma of the intra-hepatic ducts resembles carcinomas of the extra-hepatic biliary tract and is best classified with them. These groups of tumours have similar microscopic appearances and their separation, which is only based on their anatomical location in the biliary tract, is often arbitrary or impossible when there is widespread tumour involvement due to invasive spread or a wide field of origin.

It is accepted, however, that although hepatocellular and cholangiocellular carcinomas ordinarily present sharply contrasting microscopic features, some tumours may display features of a somewhat intermediate or mixed character. Such appearances are mostly due to the capacity of liver cell carcinoma to exhibit variations in microscopic structure, in particular to assume an alveolar or tubular

architecture similar to that of cholangiocellular tumours. The argument that such appearances invalidate the histogenetic distinction between these tumours becomes weak when one considers the microscopic variations which every malignant tumour may exhibit and the structural similarity which is sometimes observed in tumours of widely different origin. Carcinoma of the liver poses an additional diagnostic problem occasioned by the existence of two different tumours in the same organ. In supporting the view that hepatocellular and cholangiocellular carcinomas are structural variants of a homogeneous group of tumours, Berman (1951) argued that liver cells and bile ducts have a common embryological derivation, that the adult cells of one type can change to the other and that it is not unexpected that a similar reaction may take place in carcinoma of the liver. However, embryologists have not agreed on the details of hepatic development and the consensus of opinion is that liver cells and bile ducts have a common embryological derivation but are formed from groups of cells that have separated early in foetal life. Furthermore, it is the adult character of the tissues of origin rather than their embryological derivation which determines the behaviour of tumours and on which classification is satisfactorily based. The transformation of one type of cell to the other is a debatable point; there is no unequivocal evidence of such a change and most writers have agreed that it does not play any significant role in the regeneration of liver tissue. The view that these cells grow independently of each other is supported by experimental findings (Cameron and Oakley, 1934).

The origin of hepatocellular and cholangiocellular carcinomas from the liver cells and bile ducts respectively is abundantly shown by their microscopic appearances, their association with hyperplastic states of their parent tissues and the occasionally demonstrable transition between the tumour cells and their normal precursors. The findings in this series endorsed the importance of intra-hepatic dissemination in the development of multi-nodular tumours but indicated that a genuine multi-focal origin also occurs in many cases. The greater frequency of carcinoma in the right lobe was found to be proportionate to its bulk.

One of the main findings in the present study was the disparity between the immigrant Chinese from South China and the indigenous Chinese of Singapore in their susceptibility to develop liver cell carcinoma. The high tumour incidence in the former group was not observed among the Singapore-born Chinese and, as there is no genetic difference between them, an investigation of changes in their environment or mode of life promises to be fruitful.

SUMMARY

A summary of the pathological findings in 105 cases of carcinoma of the liver and biliary tract is presented with a review of the histogenesis, causation and distribution of these tumours. It was considered that liver cell carcinoma and carcinoma of the intra-hepatic bile ducts are heterogeneous tumours and that the latter are closely similar to carcinomas of the extra-hepatic biliary tract. It is shown that the high incidence of liver cell carcinoma among the Chinese of Singapore is restricted to the immigrants from South China.

My thanks are due to Dr. W. J. Vickers, Dr. R. H. Bland, Dr. C. Subrahmanyam and Dr. L. S. da Silva, Singapore, for the opportunity to make this study and for

permission to publish, to Mr. John Chee Kah Hoe, Mr. A. Ponnuthurai and Mr. S. Anthony for the histological preparations and to Mr. V. Nalpon for the photomicrographs.

REFERENCES

ALLEN, R. A. and LISA, J. R.—(1949) *Amer. J. Path.*, **25**, 647.
ALTHAUSEN, T. L. AND KERR, W. J.—(1927) *Endocrinology*, **11**, 377.
ARMINSKY, T. C.—(1949) *Cancer*, **2**, 379.
BERK, J. E. AND LIEBER, M. M.—(1941) *Amer. J. med. Sci.*, **202**, 708.
BERMAN, C.—(1951) 'Primary Carcinoma of the Liver.' London (H. K. Lewis).
BONNE, C.—(1935) *Amer. J. Cancer*, **25**, 811.—(1937) *Ibid.*, **30**, 435.
BRUNSCHWIG, A.—(1953) *Cancer*, **6**, 725.
CAMERON, G. R. AND OAKLEY, C. L.—(1934) *J. Path. Bact.*, **38**, 17.
CHARACHE, H.—(1939) *Amer. J. Surg.*, **43**, 96.
COPELAND, D. H. AND SALMON, W. D.—(1946) *Amer. J. Path.*, **22**, 1059.
EDMONDSON, H. A. AND STEINER, P. E.—(1954) *Cancer*, **7**, 462.
EWING, J.—(1940) 'Neoplastic Diseases,' 4th. Edn., Philadelphia (W. B. Saunders).
GILLMAN, J., GILLMAN, T. AND GILBERT, C.—(1950) *J. nat. Cancer Inst.*, **11**, 653 (Abstr.).
GOLDZIEHER, M. AND VON BOKAY, Z.—(1911) *Virchows Arch.*, **203**, 75.
GORER, P. A.—(1940) *J. Path. Bact.*, **50**, 17.
GREENE, J. M.—(1939) *Int. Abstr. Surg.*, **69**, 231.
GUSTAFSON, E. G.—(1937) *Ann. intern. Med.*, **11**, 889.
HARTZ, P. H.—(1945) *Arch. Path.*, **39**, 1.
HOEPPLI, R.—(1933) *Chin. med. J.*, **47**, 1125.
HOU, P. C.—(1955) *J. Path. Bact.*, **70**, 53.
HOYNE, R. M. AND KERNOHAN, J. W.—(1947) *Arch. intern. Med.*, **79**, 532.
KENNAWAY, E. L.—(1944) *Cancer Res.*, **4**, 571.
KIRSHBAUM, J. D. AND KOZOLL, D. D.—(1941) *Surg. Gynec. Obstet.*, **73**, 740.
KOSTER, H. AND KASMAN, L. P.—(1932) *Amer. J. Surg.*, **17**, 237.
KOUWENAAR, W.—(1932) *Geneesk. Tijdschr. Ned.-Ind.*, **72**, 382.
LIU, Y.—(1953) *Chin. med. J.*, **71**, 183.
MACCALLUM, W. G.—(1928) 'Text Book of Pathology.' 4th Edn., Philadelphia (W. B. Saunders).
MALLORY, T. B.—(1930) *New Engl. J. Med.*, **202**, 1260.—(1940) *Ibid.*, **223**, 731.
MARSHALL, J. M.—(1932) *Surg. Gynec. Obstet.*, **54**, 6.
MAXWELL, J. L.—(1928) *Chin. med. J.*, **42**, 69.
MOHARDT, J. H.—(1939) *Int. Abst. Surg.*, **69**, 440.
NEIBLING, H. A., DOCKERTY, M. B. AND WAUGH, J. M.—(1949) *Surg. Gynec. Obstet.*, **89**, 429.
OSHLAG, J. A., MARTIN, R. F. AND BINFORD, C. H.—(1945) *Amer. J. med. Sci.*, **210**, 245.
OVERTON, R. C., KADEN, VAN G. AND LIVESAY, W. R.—(1955) *Surgery*, **37**, 519.
PIRIE, J. H. H.—(1921) *Med. J. S. Afr.*, **17**, 87.
PYBUS, F. C., AND MILLER, E. W.—(1942) *Rep. Brit. Emp. Cancer Campgn.*, **19**, 42.
Report on the 1947 Census of Population. Government Publications Bureau, Singapore.
ROSENTHAL, S. R.—(1932) *Arch. Path.*, **13**, 88.
ROWEN, H. S. AND MALLORY, F. B.—(1925) *Amer. J. Path.*, **1**, 677.
SANES, S., AND MACCALLUM, J. D.—(1942) *Ibid.*, **18**, 675.
SHELDON, J. H.—(1935) 'Haemochromatosis.' London (Oxford University Press).
SHELDON, W. H. AND JAMES, D. F.—(1948) *Arch. intern. Med.*, **81**, 666.
SLYE, M.—(1916) *J. Cancer Res.*, **1**, 383 and 503.
SMITH, K. J.—(1933) *J. Lab. clin. Med.*, **18**, 915.

SNIJDERS, E. P. AND STRAUB, M.—(1923) *Trans. Far-East. Ass. trop. Med.*, 779.
STEWART, M. J.—(1922) *Brit. med. J.*, ii, 1066.—(1931) *Lancet*, ii, 565, 617, and 669.
STRONG, G. F. AND PITTS, H. H.—(1930) *Arch. intern. Med.*, **46**, 105.—(1932) *Ann. intern. Med.*, **6**, 485.
TULL, J. C.—(1932) *J. Path. Bact.*, **35**, 557.
WALSHE, J. M. AND WOLFF, H. H.—(1952) *Lancet*, ii, 1007.
WELLS, H. G.—(1903) *Amer. J. med. Sci.*, **126**, 403.
WILBUR, D. L., WOOD, D. A. AND WILLET, F. M.—(1944) *Ann. intern. Med.*, **20**, 453.
WILLIS, R. A.—(1953) ' Pathology of Tumours', 2nd Edn. London (Butterworth).
YAMIGAWA, K.—(1911) *Virchows Arch.*, **206**, 437.

CANCER AND ATHEROSCLEROSIS

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Received for publication April 4, 1956

In previous investigations the incidence of calcified atheroma in gastric and duodenal ulcers and in carcinoma of the stomach in patients over 50 years of age was examined (Elkeles, 1949, 1950, 1953). Radiography of the abdominal aorta was used to evaluate the presence or absence of calcified atheroma.

More or less advanced calcification of the abdominal aorta was found in 75 per cent of the gastric ulcer series, in 34 per cent of the duodenal ulcers and approximately the same percentage in the control series. A surprisingly low incidence of calcified atheroma (5 per cent) was found in gastric carcinoma. It seemed, therefore, of interest to extend these studies to carcinoma of other organs.

The rare occurrence of severe arteriosclerosis in cancer patients has been observed by several pathologists. Busch (1924) states that a large number of cancer patients are completely free from lipoid infiltrations and sclerosis of the arteries. Sjoeval and Wihman (1934) in their study of arteriosclerosis in Sweden point out that the low degree of arteriosclerosis in cancer cases is striking compared with the total material examined by them. Wegelin (1935) arrives at similar conclusions and finds support in a Berlin statistic which clearly demonstrates that severe arteriosclerosis is less frequent in cancer patients between 40 and 70 years of age than in the whole autopsy material of the corresponding age group. He believes in a fundamental difference of disposition between the two diseases. Casper (1932), who examined the degree of arteriosclerosis in 160 post-mortem examinations with malignant new growth and 300 controls, expressed the view that arteriosclerosis and malignant neoplasm exclude each other to some extent. In a recent study on the pathogenesis of coronary sclerosis, Lober (1953) investigated separately from the main group 121 cases who died of malignant neoplasm. The degree of coronary sclerosis in this group was found to be considerably lower than that of the main group. Wanscher, Clemmesen and Nielsen (1951) who studied the atherosclerotic lesions in 822 cancer and in 1013 control cases on post-mortem material found a pronounced difference in degree of atherosclerotic lesions between the cancer and control series.

The purpose of this paper is to present the results of an investigation in which the incidence of calcified atheroma in patients with cancer and control cases over 50 years of age was studied during life. Radiography of the abdominal aorta was found to be a valuable method for this purpose. This segment of the aorta was chosen as the test area, because the earliest and the most severe manifestations of generalized arteriosclerosis are found here. Haythorn *et al.* (1936) in their investigation on the calcium content of aortas found the heaviest calcium deposits in the abdominal portion. The radiographic method is limited to the detection

of calcified atherosclerosis, but in the vast majority of advanced lesions calcium deposits are present. According to Petersen (1952) a compact plaque of calcium about 1 cm. in diameter can usually be visualized on radiographs.

A classification of the calcified atheromatous lesions has been attempted by designating a thin linear plaque approximately 3–5 cm. in length as +, 10–12 cm. or parallel linear shadows as ++, and more extensive lesions involving almost the whole wall as +++. The abdominal aorta was radiographed in 1116 patients over 50 years of age, comprising 416 carcinoma cases, in whom the diagnosis was verified by either operation, biopsy or post-mortem examination, and 700 controls chosen at random from in- and outpatients of general hospitals.

The results are given in Tables 1 and 2.

TABLE I.—*Cancer of Various Organs*

Age.	Number of cases.	Degree of calcification of abdominal aorta on Roentgenograms. 0	+	++	+++	Per cent of calcification.
50–60	150	134	7	7	2	10·7
61–70	164	131	8	20	5	20·1
71–80	102	73	8	14	7	28·4
Total number	416					

Calcification = 18·75 per cent.

TABLE II.—*Control Cases*

Age.	Number of cases.	Degree of calcification of abdominal aorta on Roentgenograms. 0	+	++	+++	Per cent of calcification.
50–60	312	259	30	17	6	16·9
61–70	264	156	42	37	29	40·9
71–80	124	35	16	24	49	71·8
Total number	700					

Calcification = 35·7 per cent.

The investigation shows a much lower incidence of calcified atheroma in the cancer series (18·7 per cent) than in the control cases (35·7 per cent). This difference is even more pronounced in the older patients. In the 71–80 age group 71·9 per cent of the controls compared with 28·4 per cent of the cancer patients show evidence of calcified atheroma. There is also a significant difference in the occurrence of severe atheromatous lesions. +++ calcification was found in 12 per cent of the controls and in only 3·4 per cent of the cancer cases.

The low degree of atherosclerosis in cancer cases is difficult to explain. Wanscher, Clemmesen and Nielsen (1951) assume that the presence of carcinoma reduces the extension of atherosclerosis. A similar view is held by Lober (1953), who believes that the low degree of atherosclerosis in cancer patients is the result of alterations in nutrition during a prolonged terminal illness. Since he found the degree of coronary sclerosis in the cancer series about 10 years behind that of the group without malignancy, he assumes an actual regression of the atherosclerotic lesions. These theories cannot easily be refuted when solely based on post-mortem

material. The radiographic method, however, permits a study of the degree of calcified atheroma in cancer patients when anaemia or cachexia have not yet developed. The results of the present investigation do not support the concept that the low degree of atherosclerosis in cancer patients is due to the process of wasting. It may be added that according to Wilens (1947) a low nutritional state over a prolonged period may lead to a reduction in the amount of lipid contained in the intimal lesions but not to any significant diminution of the other features of the atherosclerotic process.

In the course of this investigation it became evident that a true picture of the cancer-atherosclerosis relationship can only be obtained by studying the incidence of calcified atheroma in cancer of different organs. An attempt has, therefore, been made to analyse this relationship in some of the more common sites of cancer.

The rare occurrence of calcification in the abdominal aorta in cancer of the stomach is striking. Only six out of 91 cases (6·6 per cent) show evidence of calcified atheroma. It has been suggested that an inherited element plays a part in the susceptibility to gastric carcinoma (Aird, Bentall and Fraser Roberts, 1953; Gorer, 1938). The present findings seem to be in conformity with this concept but suggest a genetic element for the susceptibility to the more severe type of atherosclerosis as well. It seems that these two genetic factors occur rarely together in the same individual.

A low degree of atherosclerosis has also been found in carcinoma of the sex glands. Nine out of 65 cases of carcinoma of the breast (13·8 per cent) and 3 out of 31 cases of carcinoma of the prostrate (9·7 per cent) show evidence of calcified atheroma. In the latter the number of cases is rather small, but it is significant that 26 of these occurred in the age group of 61–80. The slightly higher percentage of calcification in carcinoma of the breast can perhaps be explained by the observation that atherosclerosis in women after the menopause is more pronounced than in men of the corresponding age group (Petersen, 1952).

In contrast to cancer of the stomach and of the sex organs, severe atheroma is at least as common in cancer of the respiratory tract as in the control series. Eighteen out of 48 cases (37·5 per cent) show calcification of the abdominal aorta mainly of an advanced degree.

With our present limited knowledge of the origin of cancer any attempt to explain the results of this study are bound to be somewhat speculative. The evidence of this investigation points to the possibility that cancers, which are influenced by genetic elements or are hormone dependent, show a low incidence of calcified atheroma. Cancers predominantly caused by exogenous carcinogenic agents show the same percentage of calcified atheroma as the control cases.

Thus, individuals with marked atherosclerosis are less likely to develop carcinoma of certain organs, e.g. of the stomach, breast, prostate.

SUMMARY

Radiography of the abdominal aorta has been found to be a valuable method for recognizing calcified atheroma during life. The abdominal aorta was radiographed in 1116 patients over 50 years of age, comprising 416 cases of cancer of various organs and 700 controls.

A low incidence of calcified atheroma has been found in the cancer cases (18·7 per cent) compared with the control series (35·7 per cent). This difference

is even more pronounced in the 71–80 age group, 28·4 per cent against 71·9 per cent.

Calcified atheroma is rare in carcinoma of the stomach and its incidence is low in carcinoma of the prostate and breast. The percentage of calcified atheroma in cancer of the respiratory tract does not differ from that of the control series.

The hypothesis is proposed that the incidence of calcified atheroma is low in cancers which are influenced by genetic factors or are hormone dependent. The incidence of calcified atheroma in cancers predominantly caused by exogenous carcinogenic agents does not differ from the control series.

Individuals with marked atherosclerosis are less likely to develop cancer of certain organs, e.g. of the stomach, breast, prostate.

REFERENCES

AIRD, I., BENTALL, H. H. AND FRASER ROBERTS, J. A.—(1953) *Brit. med. J.*, i, 799.
BUSCH, M.—(1924) *Zbl. allg. Path. path. Anat.*, **34**, 614.
CASPER, J.—(1932) *Z. Krebsforsch.*, **36**, 354.
ELKELES, A.—(1949) *Brit. J. Radiol.*, **22**, 280.—(1950) *Int. Congr. Radiol.*, p. 78.—(1953) *Amer. J. Roentgenol.*, **70**, 797.
GORER, P. A.—(1938) *Ann. Eugen., Lond.*, **8**, 219.
HAYTHORN, S. R., TAYLOR, F. A., WHITEHILL CRAGO, H. AND BURRIER, A. Z.—(1936) *Amer. J. Path.* **12**, 283.
LOBER, P. A.—(1953) *Arch. Path.*, **55**, 357.
PETERSEN, G. F.—(1952) *Acta Radiol., Stockh.*, **37**, 356.
SJOEVAL, H. AND WIHMAN, G.—(1934) *Acta path. microbiol. scand.*, Suppl. 20, 1.
WANSCHER, O., CLEMMESEN, J. AND NIELSEN, A. —(1951) *Brit. J. Cancer*, **5**, 172.
WEGELIN, C.—(1935) *Schweiz. Med. Jb.*, **99**.
WILENS, S. L.—(1947) *Amer. J. Path.*, **23**, 793.

MALIGNANT DISEASE IN OLD AGE

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Received for publication April 12, 1956

In spite of the growing importance of geriatrics it is evident on reviewing the literature that one aspect of the problem, namely the pathology of old age, has received but scant attention. Howell and Piggot (1951) declared that there was as yet little knowledge concerning the morbid anatomy found in aged persons and Medalia and White (1952) could trace only one reference related to pathological observations in old persons. This lack of information may be partly due to the realisation that post mortem statistics are often based on relatively selected material and may not reflect the true incidence of various diseases in the older age-groups. Nevertheless analysis of such material is essential for the establishment of a sound basis for geriatric pathology which will also serve as a standard for comparison with similar studies in other age groups.

The purpose of this communication, therefore, is to present an analysis of the post mortem findings in patients dying over the age of 70, dealing in particular with the various forms of malignant disease encountered, and their importance as a cause of death.

MATERIAL

The cases studied were derived from two general hospitals, the Royal Victoria and City Hospital, Belfast, the latter having a geriatric unit. Post-mortem examination was performed on 1,300 patients over the age of 70, 757 being males and 543 females. 1036 were between the ages of 70 and 80, 252 between 80 and 90, and 12 between 90 and 100.

The first table gives a general indication of the distribution of deaths according to the systems involved.

TABLE I

	No. of deaths in various age groups.
Cardiovascular system	277 (21%)
Alimentary system	235 (18%)
Respiratory system	174 (13%)
Central nervous system	157 (12%)
Urinary system	133 (10%)
Liver and gall bladder	75 (6%)
Trauma	67 (5%)
Peripheral vascular disease	60 (5%)
Haemopoietic	30 (2%)
Pancreas	22 (2%)
Uncertain	21 (2%)
Miscellaneous	49 (4%)
	1300

Disease of the cardiovascular system was the commonest cause of death in the entire group, 277 patients (21 per cent) dying from heart disease. Malignant disease was the second major cause of death, accounting for 261 fatalities (20 per cent). Carcinoma was an incidental finding in a further 64 patients thus raising the total incidence of malignancy to 25 per cent. 27·6 per cent males had malignant tumours compared with 21 per cent females. The incidence of malignancy in the 70–80 age group was 24 per cent, in the 80–90 group 29 per cent, and in the 90–100 group, 25 per cent. There is a close correlation between these findings and those of Howell and Piggot (1952) in their series of autopsies on patients dying between the age of 75 and 79. They found cardiovascular disorders foremost as a cause of death, with cancer second, and tumours of the large intestine, as in this series, the most frequent of all malignancies.

An indication of the sites most frequently involved in this series is given in Table II.

TABLE II

Site.	Total number of tumours.	Number as cause of death.
Large intestine (including rectum)	73	67
Prostate	47	27
Stomach	42	29
Lungs	32	27
Oesophagus	17	17
Bladder	16	14
Kidney	15	11
Pancreas	14	14

Malignant disease of the alimentary tract

Carcinoma of the large intestine was the commonest tumour found in this series, occurring in 73 cases and proving fatal in 67. Its great frequency contributed largely to the importance of disease of the alimentary tract as a cause of death. Males were affected more frequently than females (41 M. : 32 F.) but in view of the greater number of males than females in the series these figures actually represent a slight preponderance of this form of carcinoma in the female. In both sexes there was a relatively higher incidence in the 80–90 age group than in the 70–80 group.

In 12 cases the tumour was situated in the caecum, in 49 in the colon, and in 12 in the rectum. The pelvic colon was the most frequent site being involved in 16 cases. More than one-third of the patients died following operative treatment, 16 undergoing resection and 7 colostomy. In the unoperated group acute obstruction was a common termination occurring in 24 cases, and in 12 of these perforation and peritonitis had resulted. In 4 the perforation had developed at the site of the tumour and in 8 proximally. In colonic growths the caecum ruptured in 4 instances.

A study of metastatic spread revealed one or two interesting features. In a surprisingly high number of cases, 35/73, no metastases were discovered. However operative resection had been performed in 16 of these, 6 were incidental tumours and 10 of the 12 caecal growths showed a remarkable absence of metastases. Extensive lymph node involvement was found in 16 cases, and metastases to the liver in 15. Deposits in the lungs were discovered in only 3 cases, and in bone in 2. Carcinomatosis peritonei occurred only 3 times. One case was of particular

interest in illustrating the long survival which may occur in carcinoma of the large bowel. A male of 78 years had a right hemicolectomy for carcinoma of the colon 16 years previously. A recurrence of tumour developed at the site of anastomosis and was resected 10 years later. He died 6 years after this operation with intestinal obstruction due to adhesions and no residual tumour and no metastases were found.

The findings in this group of elderly patients show that carcinoma of the large bowel most often proved fatal by reason of its local complications, frequently giving rise to obstruction with or without perforation and peritonitis. The operative mortality for resection was also high. Dissemination of the tumour was not so important a factor in causing death since less than 25 per cent of cases showed metastases in the liver, peritoneal spread was rare, and pulmonary involvement was seldom seen.

Carcinoma of the stomach was the third commonest tumour, with 42 examples, 29 proving fatal and 13 incidental. The sex ratio was 28 M.: 14 F. Thirty-five occurred in the 70–80 age group, and 7 in the 80–90 group. Only 3 patients received operative treatment, to which they all succumbed. One patient had Wernicke's encephalopathy. In the 26 fatal cases (without operation) 21 had lymph node metastases, 13 had metastases in the liver, 5 in the lungs, 3 in bone, and 8 had carcinomatosis peritonei. In the incidental groups of 13 cases only 2 showed lymph node metastases. The findings in this group differ considerably from those in the colonic group. Operative treatment was rare and was poorly tolerated. Dissemination of the tumour was more frequent and widespread. This was not entirely due to a delay in seeking treatment for in no case was the history longer than 2 years and in many, symptoms had been present for only some weeks. Another striking feature, of uncertain significance, was the presence of other major pathological conditions in many patients with gastric carcinoma, whereas the colonic group was remarkably free from other diseases. On the whole, the occurrence of so many unsuspected gastric tumours, and the degree of spread in the fatal cases in spite of a short history, would suggest that carcinoma of the stomach is often well established before symptoms arise.

Carcinoma of the oesophagus was 5th in frequency, occurring in 17 patients and proving fatal in all. The sex ratio was 14 M.: 3 F. Thirteen occurred in the 70–80 age group, and 4 in the 80–90 group. Only one tumour involved the upper end of the oesophagus, 8 the level of the bifurcation of the trachea, and 8 the distal third. The tumour remained well localised in a high percentage of cases, 11 with no metastases, 6 with lymph node metastases and only 3 with dissemination to lungs, liver, kidney and bone. Five patients died post-operatively and most of the others from local complications such as mediastinitis, bronchopneumonia and empyema. These figures do not represent the true incidence of this disease in elderly patients, as the number of cases studied depends largely on local surgical interest and other factors. There are no very accurate figures indicating its frequency in the elderly, but Howell and Piggot (1952) found it 4th in the late 70's and over 80 age group. In most recorded series the preponderance in the male sex is striking, and apart from local lymph node metastases, widespread dissemination appears to occur in only a small proportion of cases.

This short analysis of new growths of the alimentary tract indicates that in this series of elderly patients carcinoma of the large intestine was the commonest tumour encountered, gastric carcinoma was third, and oesophageal carcinoma

fifth, these together constituting just over 40 per cent of all malignancies in the series.

Carcinoma of the prostate

Carcinoma of the prostate was the second most frequent tumour, but as a cause of death from carcinoma it ranked third. There were 47 cases of frank carcinoma, 27 of whom died as a result of the disease. Nine patients died post-operatively and in the remainder death was generally due to ascending infection and uraemia. Seven of the fatal cases were recurrences 2–8 years after prostatectomy. As regards dissemination in the fatal cases lymph node metastases were found in 8, pulmonary in 6, hepatic in 2, and bone in 6. Of the remaining 20 incidental tumours wide infiltration of the gland by carcinoma was noted, often with perineural lymphatic and lymph node involvement.

In addition to these 47 examples of carcinoma of the prostate, there was focal carcinomatous change in another 38 cases, which brings the total incidence of prostatic carcinoma in the series to 11 per cent. An even higher incidence (14 per cent) was noted by Rich (1935) in 292 consecutive autopsies on men over the age of 50, and Moore (1935) discovered prostatic carcinoma with a frequency which increased with age, mounting to 29 per cent, in the 9th decade. An increase with age was also experienced in this series, with 4·6 per cent of tumours occurring in the 70–80 age group, compared with 14·6 per cent in the 80–90 group. This confirms Willis' statement (1953) that most deaths from this disease occur in the 8th decade.

Carcinoma of the lung

Carcinoma of the lung was the 4th commonest tumour, there being 32 cases. It accounted for 27 deaths. As in all recorded series the sex ratio was predominantly male, 22 M.: 10 F. Twenty-five cases occurred in the 70–80 age group, 6 in the 80–90 group, and 1 in a patient aged 90. Considering the numbers in each group there was no waning incidence with age though in most series the peak has been noted to occur in the 6th decade. Five of the tumours were incidental and in addition to these, 5 microscopic carcinomas were found in scars. There was also 1 pleural endothelioma. Histologically 16 were oat cell, 10 were squamous, and 6 adenocarcinoma. Apart from hilar node involvement which was noted in more than three-quarters of the cases, the liver contained metastases in 13, adrenals in 6, and bone and brain in 5.

Other tumours

Other tumours which occurred with some frequency were carcinoma of the bladder, kidney and pancreas. There were 16 malignant bladder tumours, all of squamous type except for 1 round-cell sarcoma. Males predominated with a 12 M.: 4 F. incidence. Fourteen tumours occurred in the 70–80 age group, and 2 in the 80–90 group. The cause of death was chiefly ureteric obstruction with ascending infection and uraemia. Three patients died shortly after ureteric transplantation and 1 survived a year. Distant metastases occurred in only 2 cases.

Of the 15 malignant tumours of the kidney, 12 were hypernephromas, 2 carcinomas of the pelvis, and 1 myosarcoma. One of the pelvic tumours had eroded the wall of the duodenum establishing a wide fistulous communication,

with duodenal contents passing freely into the renal pelvis. Four of the hypernephromas were incidental findings, and one an extremely small tumour only 1 cm. in size. Again males predominated, 11 M.: 4 F. Pulmonary metastases were found in only 2 cases and vertebral and epidural space in 1.

There were 14 cases of pancreatic carcinoma, fatal in all. The sex incidence was 9 M.: 5 F. Twelve of the tumours were situated in the head, 1 in body and tail, and 1 in tail. Nine patients had jaundice and there was a very high incidence of metastases, with liver involvement in 11, and pulmonary in 8. Death was due to pulmonary embolism from peripheral venous thrombosis in 2 cases, and in a third case the popliteal and femoral veins showed thrombosis secondary to actual tumour invasion of their walls.

Of the remaining tumours there were 8 examples of carcinoma of the breast, 1 occurring in a male, and 7 uterine tumours of which 5 were carcinomas and 2 sarcomas. There were 8 malignant tumours arising in the bile ducts, and 7 in the gall bladder. This was the only growth common to both sexes which showed a marked predominance in the female (5 F.: 2 M.). Carcinoma of the liver was encountered 4 times, in each instance superimposed on nodular hyperplasia. Brain tumours were infrequent only 4 glioblastomas being found, all in males, and 1 astrocytoma in a female. There were 4 cervical carcinomas, 2 of vulva, 1 of ovary, 1 of thyroid, 1 of larynx and 1 of adrenal cortex. Reticulum sarcoma and lymphosarcoma were each encountered twice, and retroperitoneal sarcoma and melanoma once. There were 8 examples of lymphatic leukaemia, and 2 of multiple myeloma. No tumour of testis or of bone was found in the series.

Multiple malignancies

The frequency of multiple malignancies in the same patient was not striking. Apart from focal carcinoma of the prostate of which there were 11 cases associated with malignant tumours in other sites there were only 4 genuine examples: carcinoma of the lung and bladder, myosarcoma of the kidney and carcinoma of the stomach, glioblastoma and carcinoma of the stomach, hypernephroma and carcinoma of stomach. Lymphatic leukaemia occurred in association with carcinoma of the lung in 1 case, and with an extensive carcinoma of the prostate in another.

CONCLUSIONS

In this series of 1300 post mortems on patients dying over the age of 70, heart disease ranked first and cancer second as a cause of death. Twenty-five per cent of patients in the series had malignant disease and this proved fatal in 20 per cent. Males were affected more frequently than females, confirming the findings of other workers on the sex incidence of carcinoma in later life. The incidence of tumours rose slightly in the 8th decade as compared with the 7th, but this was partly accounted for by the increasing incidence of carcinoma of the prostate with age.

The commonest site for a malignant tumour was the large intestine, 22 per cent of all tumours occurring in this situation. Metastatic spread was a much less marked feature of this tumour than of carcinoma of the stomach. The second most common tumour was carcinoma of the prostate, accounting for 14 per cent of all malignancies. When focal carcinomatous change was included, the total

incidence of malignant disease of the prostate rose to 11 per cent of the males in the series.

Tumours of the stomach, lungs, oesophagus, bladder, kidney and pancreas followed in that frequency, and tumours which were rare were those of cervix and ovary, thyroid, and melanoma. Nevertheless most sites were represented but tumours of bone and testis were not encountered. The incidence of multiple malignancies in the same patient was not striking, only 4 examples being found.

Of all the malignant tumours discovered 20 per cent were incidental in that they were unsuspected and did not contribute materially to the death of the patient. Such a finding emphasises the importance of complete post mortem examination as the only means of determining the true incidence of any tumour.

Thanks are due to the Medical Staff of the City and Royal Victoria Hospitals, Belfast, for access to the clinical records, and to the Northern Ireland Hospitals Authority for a research grant to this Department.

REFERENCES

HOWELL, T. H. AND PIGGOT, A. P.—(1951) *Geriatrics*, **6**, 85.—(1952) *Ibid.*, **7**, 137.
MEDALIA, L. S. AND WHITE, P. D.—(1952) *J. Amer. med. Ass.*, **149**, 1433.
MOORE, R. A.—(1935) *J. Urol.*, **33**, 224.
RICH, A. R.—(1935) *Ibid.*, **33**, 215.
WILLIS, R. A.—(1953) ' Pathology of Tumours.' London (Butterworths), p. 586.

CANCER DEATH RATES IN JAPAN CONTRASTED WITH THOSE IN ENGLAND AND WALES AND CANADA

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Received for publication April 14, 1956

COMPARISONS between death rates from cancer of particular organs in countries such as Denmark, Holland, Britain, Canada and the United States of America have revealed such a measure of agreement in many instances that pronounced differences, when they do appear between countries having good diagnostic and registration services, are worthy of attention. If such differences cannot be explained by variations in terminology, classification, completeness of ascertainment, accuracy of death certification or facilities for treatment, a search for the underlying reasons for them may lead in the end to discovery of causes of the forms of cancer concerned. For that reason it is worth while to call the attention of research workers to pronounced international differences in rates which appear to satisfy the above conditions, even though no explanation can yet be suggested.

For a long time it had been noticed that Japan's death rate from breast cancer appeared to be very small in comparison with the rates in western countries, but it was not until about 1950 that serious attention began to be paid to this. In that year the deaths of females from all forms of cancer registered in Japan numbered 31,758, of which 1,419 or 4½ per cent, were assigned to breast cancer. The corresponding proportion in England and Wales was 19 per cent. In a report to the World Health Organisation Training Course on Vital and Health Statistics for South West Pacific Areas in 1952, Professor Mitsuo Segi, by using the population of Japan by age groups in that year as standard, calculated age-adjusted death rates in 1949 from breast cancer in females in 8 countries to be as follows :

Canada 19·3 ; New Zealand 19·0 ; England and Wales 18·7 ; U.S.A. and Netherlands 18·5 ; Denmark 17·9 ; Australia 17·2 ; Japan 3·3. This was commented upon in several papers read to the 5th meeting of the International Society of Geographical Pathology at Washington D.C. in 1954 (*Transactions*, 1955), and no reason has been suggested why understatement of breast cancer as underlying cause of death should be appreciably greater in Japan than in the other countries. Thus, Maisin and Langerock, from their analysis of the documentation on Racial Factors (1955), concluded that " in certain countries, as e.g. Japan, where we have every reason to believe that the documents at our disposal are especially well proved and studied, we are certain that cancer of the breast is particularly low . . . ". Statistics of cause of death in recent years in Japan in respect of persons dying in early and middle life must be regarded as comparable in their reliability with those of countries such as Britain, Norway, Holland and Canada, although this is probably not true of rates at ages after 70.

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Justification for this qualification relating to advanced ages is to be found in the series of death rates from all forms of cancer combined in Table 46 of the World Health Organisation's report on Annual Epidemiological and Vital Statistics (1955), where rates for year 1952 at 5-year age groups are given for 21 countries. Perhaps the most satisfactory measure of comparison between different countries for cancer is the Equivalent Average Death Rate at ages 35–64, that is to say the arithmetic mean of the 6 rates at 5-year age groups between 35 and 65. According to this measure Japan ranks 9th for males and 15th for females when the 21 countries are arranged in descending order of the rate, as may be seen below, the rates being per 100,000 distributed equally over ages 35 to 64.

Males.—Finland 312 ; Scotland 291 ; Austria 275 ; England and Wales 269 ; Germany (Federal Republic) 260 ; Switzerland 240 ; France 234 ; Northern Ireland 225 ; *Japan* 223 ; Italy 213 ; U.S.A. (white) 212 ; Holland 209 ; Denmark 206 ; Canada 198 ; Australia 182 ; Norway 180 ; New Zealand 170 ; Sweden 164 ; Israel (Jewish) 157 ; Portugal 139 ; Ceylon 47 ;

Females.—Denmark 225 ; Scotland 221 ; Austria 218 ; Germany (F.R.) 215 ; Northern Ireland 214 ; Holland 210 ; England and Wales 209 ; Canada 207 ; U.S.A. (white) 206 ; Switzerland 204 ; New Zealand 204 ; Finland 203 ; Sweden 199 ; Israel (Jewish) 196 ; *Japan* 193 ; Norway 184 ; France 176 ; Australia 171 ; Italy 171 ; Portugal 125 ; Ceylon 52.

Japan's rates for malignant neoplasms as a whole during the middle period of life are evidently neither very high nor very low compared with countries of Europe and North America. The male rate is higher than in Australasia, and the female rate lies between those of Australia and New Zealand.

At ages above 70, however, Japan's ranking falls considerably ; for example at ages 70–74 it appears from the W.H.O. figures that the rates rank 18th amongst the 21 countries for each sex. It is most unlikely that this means a real fall in incidence at advanced ages in Japan ; it suggests rather that certification of cancer becomes incomplete amongst old people, and consequently it is best to confine attention to death rates in middle life when making comparisons with other countries, In Tables II and V the rates at ages over 70, though included, are shown in italics.

Apart from records derived from death registration, morbidity rates from breast cancer obtained in Miyagi Prefecture have been compared by Segi (1955) with similar rates of incidence at the same ages in Denmark and Manitoba, and these show at ages 40–49 a rate of 29 per 100,000 in contrast with 75 in Denmark and 115 in Manitoba, at 50–59 a rate of 48 against 108 and 135, and at 60–69 a rate of 31 against 167 and 153.

A comprehensive analysis has now been made by the Department of Public Health at Tohoku University (Segi *et al.*, 1955) of death rates from all forms of cancer during the period 1900 to 1954 in Japan, and since this shows some pronounced differences for a number of sites of cancer, it is advisable to set out some of the salient facts for study and discussion. In the present paper comparison has been made first between standardised death rates in Japan, England and Wales and Canada in two periods, using for convenience the 1951 age distribution in Canada as standard throughout. The Canadian rates are taken from a useful recent report on mortality trends by the National Cancer Institute of Canada (1955).

More detailed comparisons between age-specific rates in Japan and England and Wales are then made, the English rates being calculated from data in the Registrar General's Statistical Reviews (correcting 1936–40 rates for changes in rules for selecting the underlying cause of death).

Standardised death rates in Japan, Canada, England and Wales

Table I compares the rates in Japan and Canada in 1948 with those in England and Wales in a 5-year period centred at 1948, for the buccal cavity and pharynx, oesophagus, stomach, intestine, rectum, larynx and lung, breast and uterus, the numbers in parentheses being the categories in the new International Classification issued in that year. Similar rates are given for 1952 (England and Wales, 1952–54), and all these rates, standardised by applying the specific rates at 13 age groups to Canada's census population of 1951, are fully corrected for the effects of differing age constitution of the populations. It is not possible to separate cervix uteri in the Japanese statistics at present owing to the large numbers of deaths classed to " uterus " without further definition on death certificates. The inclusion of duodenum with stomach in the Japanese rates for 1948 has no appreciable effect on the figures.

TABLE I.—*Comparison between Standardised Death Rates* from Cancer of certain sites in Japan, Canada and England and Wales*

	Mouth and pharynx (140–8).	Oeso-phagus (150).	Stomach (151).	Intes-tine (152–3).	Rectum (154).	Larynx and lung (161–3).	Breast (170).	Uterus (171–4).
Males								
Japan,								
1948	2·6	—	53·4†	—	—	3·9	—	—
1952	1·3	6·5	62·2	2·1	3·8	4·9	—	—
Canada,								
1948	6·1	—	27·9	14·9	8·3	13·9	—	—
1952	4·9	—	28·0	16·0	7·2	19·2	—	—
England and Wales								
1946—50	—	5·2	27·5	16·4	13·2	32·0	—	—
1952–54	4·6	4·6	26·5	14·2	11·1	45·3	—	—
Females								
Japan,								
1948	1·5	—	33·1†	—	—	—	3·9	23·6
1952	0·6	2·5	37·5	2·6	3·3	2·0	4·0	22·1
Canada,								
1948	1·3	—	16·1	18·7	5·5	3·7	23·5	16·6
1952	1·3	—	14·0	17·8	5·2	4·0	23·2	14·4
England and Wales								
1946–50	—	2·4	18·5	17·9	7·6	6·2	24·3	13·1
1952—54	1·7	2·4	17·0	15·4	6·7	7·0	24·0	11·5

* Per 100,000 living, using as standard population that of Canada in 1951.
† Including duodenum.

The Canadian rates agree closely with the English rates for the mouth and pharynx, stomach, intestine and breast, but for rectal and respiratory cancers they are much lower, and for uterine cancer rather higher. The Japanese rates are about double those of either country for stomach cancer, and for the oesophagus the male rate in 1952 is 1·4 times that in England and Wales. In contrast with this, the intestinal rates in Japan are less than one-sixth of the English and

Canadian rates, and for the rectum, mouth and pharynx they are also lower. For the whole digestive tract Japan's rates in 1952 are 76 for males and 46 for females, compared with 61 and 43 respectively in England and Wales, but the contribution of intestine with rectum to these totals is only 6 for each sex in Japan compared with about 23 in the other countries. No reason for the high gastric and low intestinal rates in Japan is known, but it might well lie in the pronounced differences between the national dietaries. The low rates for respiratory cancers are seen below to be due to the lung and bronchus, and are scarcely surprising in view of differences in smoking and atmospheric pollution.

Breast cancer rates did not change appreciably between 1938 and 1952 in any of the three countries, and the Japanese rate has remained at about one-sixth of the Canadian and English rates. When the breast and uterus are studied in detail of age, however, the comparison is not as simple as these figures suggest.

Mortality at different ages from breast, uterus, stomach and other cancer

Table II contrasts the death rates from these kinds of cancer at different ages in Japan in 1938 and 1952 with the corresponding mean annual rates in England

TABLE II.—*Comparison between Age-specific Death Rates per Million from Cancer in Japan and those in England and Wales*

Age group.	Breast, Female.		Uterus, Female.		Stomach. Male.		Stomach. Female.		Other cancer.* Male.		Other cancer.* Female.	
	Jap.	E. & W.	Jap.	E. & W.	Jap.	E. & W.	Jap.	E. & W.	Jap.	E. & W.	Jap.	E. & W.
Japan, 1938, with England and Wales, 1936–40												
0–	0	0	2	1	2	1	3	1	16	41	11	31
25–	8	28	93	21	38	21	50	18	57	125	49	96
35–	22	123	317	97	154	76	160	50	121	252	139	220
40–	45	263	503	209	321	156	249	86	235	494	210	382
45–	67	455	608	336	693	307	401	165	449	874	373	647
50–	87	622	737	448	1274	551	665	278	832	1596	544	1072
55–	98	778	788	522	2190	872	1173	464	1371	2739	805	1553
60–	109	927	806	591	3224	1344	1603	770	1966	4480	1034	2284
65–	111	1049	721	668	3579	1935	1936	1234	2334	6620	1250	3255
70–	*155*	1239	*633*	719	*3852*	2503	*2082*	1773	*2730*	9045	*1459*	4611
75–		1522		727		2993		2253		11458		5192
80–	*191*	1834	*301*	755	*2278*	2675	*1322*	2267	*2466*	12027	*1154*	6636
85+		2548		625		1891		2251		11475		7832
Japan, 1952, with England and Wales, 1952–54												
0–	0	0	2	1	2	1	2	1	20	52	16	37
25–	11	32	53	23	56	14	54	12	43	121	55	95
35–	41	138	198	66	170	49	171	33	126	280	120	201
40–	74	296	365	116	373	117	288	63	231	565	237	401
45–	81	463	555	181	700	254	418	121	463	1121	342	677
50–	116	565	707	289	1312	449	666	205	828	2259	558	1103
55–	138	709	750	392	2194	731	1092	343	1348	3765	857	1574
60–	132	836	807	467	3288	1212	1546	527	2094	5374	1155	2244
65–	117	1012	842	509	4552	1774	2269	883	2949	7520	1588	3026
70–	*140*	1173	*721*	546	*5119*	2337	*2666*	1372	*3191*	9690	*1856*	4035
75–	*171*	1422	*595*	654	*4574*	2943	*2660*	1983	*3242*	12748	*1888*	5544
80–	*182*	1789	*465*	650	*2869*	3103	*1858*	2548	*2469*	14512	*1568*	7302
85+		2360		652		2566		2595				

* Excluding leukaemia and Hodgkin's disease. The Japanese rates in 1938 for stomach include duodenum.

and Wales in 1936–40 and 1952–54. For stomach the Japanese rates in 1952 are 4 or 5 times the English at ages under 45, and the ratio then falls to about 3 at 60. After 70 Japan's rates decline. probably owing to incomplete ascertainment or certification. For uterus the Japanese figure reaches a maximum about age 60 in 1938 and about age 65 in 1952, whereas the English rates continue to increase with advancing age ; and the ratio is about 3 to 1 at 35, falling by age 60 to 1·4 in 1938 and to 1·7 in 1952. For breast cancer death rates in both countries increase throughout life, Japan's rates being a third to a quarter of the English rates in early life and only one-ninth in late life. For all other cancer the ratio up to age 70 is about one-half.

Table III shows the trend of the percentage ratios as age advances from 25 to 70, and it is apparent that combined mortality from breast and uterine cancer in

TABLE III.—*Death Rates of Women in Japan from Cancer of Breast, Uterus, Stomach, Intestine and All Other Sites at Various Ages, expressed as percentages of the rates in England and Wales,* 1938 *and* 1952*

	25–	35–	40–	45–	50–	55–	60–	65–69
Breast—								
1938	29	18	17	15	14	13	12	11
1952	34	30	25	17	21	19	16	12
Uterus—								
1938	443	327	241	181	165	151	136	108
1952	230	300	315	307	245	191	173	165
Breast and uterus—								
1938	206	154	116	85	77	68	60	48
1952	116	117	107	99	96	81	72	63
Stomach—								
1938	278	320	290	243	239	253	208	157
1952	450	518	457	345	325	318	293	257
Intestine and rectum—								
1952	47	44	45	36	36	39	35	37
All other sites*—								
1952	58	60	59	51	51	54	51	52

* England and Wales rates used are for 1936–40 and 1952–54. " All other sites " exclude leukaemia and Hodgkin's disease.

1938 was much higher in Japan than in England and Wales up to age 45, but at ages thereafter the percentage ratio fell below 100, decreasing steadily to about 50 by age 65. In 1952 the percentage declined with advancing age from 117 at ages before 40 to 63 at ages 65–69.

Table IV estimates the rates of dying from breast and uterine cancer during the 15 years from 1937 to 1952 experienced by two " cohorts " of women who were aged 30–34 and 45–49 in 1937. In Japan each 100,000 in the younger group lost 59 by breast and 466 by uterine cancer, or 525 by the combined causes, whereas in England and Wales the same number lost 309 by breast and 156 by uterine cancer, or 465 by the combined causes. During the reproductive period Japan's higher uterine mortality more than counteracted the low breast risk, and no advantage began to accrue until after 45. In the next 15 years of life each 100,000 Japanese women starting at ages 45–49 lost 135 by breast and 987 by uterine cancer, or 1122 by the combined causes, whereas the same number of English

TABLE IV.—*Estimated Proportions of Women Aged* 30–34 *and* 45–49 *in* 1937 *who died during the next* 15 *years of Breast and Uterine Cancer in Japan and in England and Wales*

	Aged 30–34 in 1937.		Aged 45–59 in 1937.	
	Japan.	Eng. & Wales.	Japan.	Eng. & Wales.
Estimated population in 1937 (thousands)	2,326	1,731	1,530	1,427
Estimated deaths in the cohort by 1952 from :				
Breast cancer	1,383	5,352	2,083	13,141
Uterine cancer	10,853	2,693	15,096	8,804
Combined causes	12,236	8,045	17,179	21,945
Proportion per 100,000 alive in 1937 who died in 15 years from :				
Breast cancer	59	309	135	921
Uterine cancer	466	156	987	617
Combined causes	525	465	1,122	1,537

women lost 921 by breast and 617 by uterine cancer, or 1538 in all. The total expectation of dying of breast or uterine cancer during the next 30 years amongst 1000 women aged 30 must be about 20 in Japan and 22 in England and Wales, the lower risk from breast cancer being almost compensated by the higher risk from uterine cancer. Realisation of this must modify ideas about the effect of prolonged lactation on the incidence of breast cancer particularly in view of recent evidence that the important factor influencing the cervix cancer risk is early marriage rather than number of parturitions (Wynder *et al.*, 1954 ; Stocks, 1955).

Mortality at different ages from mouth, oesophagus, intestine, larynx, and lung cancers, and leukaemia

Table V compares the death rates in Japan in 1952 at various ages with the corresponding mean annual rates in England and Wales in 1952–54 for parts of the digestive tract, larynx and lung cancer, and leukaemia. Between ages 50 and 70 Japanese rates in men are about half the English rates for the buccal cavity and pharynx, and are about double the English rates for the oesophagus. For the intestine and rectum Japan's rates are about one-third of those in England and Wales for men between ages 40 and 60, and the ratio then decreases, whilst for women (as Table III shows) the ratio remains around 36 per cent from age 40 to 70. For the larynx the figures are similar in the two countries up to age 70, but for lung cancer registered mortality of men in Japan is less than one-tenth of the English rates at most ages, and amongst women the ratio is between one-half and one-third. Leukaemia rates change little with age in Japan, and they fail to show the increase from age 50 onwards which is such a pronounced feature of the English statistics.

SUMMARY

Japanese mortality from malignant neoplasms as a whole ranks 9th for men and 15th for women amongst the equivalent average death rates at ages 35–64 of 21 countries when arranged in descending order. The statistics for separate forms of cancer up to age 70 may with safety be compared with those of other

TABLE V.—*Comparison between Age-specific Death Rates Per Million in Japan, 1952, and in England and Wales, 1952–54 from Cancer of Digestive and Respiratory Organs and Leukaemia*

Age group.	Mouth and pharynx (140–8). Males.		Oesophagus (150). Males.		Intestine and rectum (152–54). Males.		Larynx (161). Males.		Lung and bronchus (162–3). Males.	
	Japan.	E. & W.	Japan.	E. & W.	Japan.	E. & W.	Japan.	E. & W.	Japan.	E. & W.
0–	0·2	2	0·1	0	0·8	1·2	0·1	0·1	0·7	2·1
25–	2	2	1·2	1·2	7	17	0·5	0·2	5	26
35–	4	7	5	5	25	45	1·2	1·8	6	97
40–	9	11	15	11	38	91	6	5	21	249
45–	12	19	45	20	53	155	18	13	43	577
50–	22	47	95	53	109	284	32	33	70	1259
55–	40	92	208	99	176	511	57	75	114	2002
60–	72	148	362	170	262	854	85	101	181	2543
65–	78	170	550	277	423	1514	151	156	236	2922
70–	*100*	491	*602*	451	*515*	2443	*146*	225	*219*	2592
75–	*131*	799	*587*	699	*521*	3825	*125*	294	*190*	2072
80+	*97*	1072	*415*	848	*428*	4719	*83*	357	*110*	1267

	Leukaemia (204). Males.		Leukaemia (204). Females.		Intestine and rectum (152–54). Females.		Larynx (161). Females.		Lung and bronchus (162–3). Females.	
	Japan.	E. & W.	Japan.	E. & W.	Japan.	E. & W.	Japan.	E. & W.	Japan.	E. & W.
0–	20	33	14	25	0·5	0·9	0·1	0·1	0·7	0·6
25–	14	20	12	15	8	17	1·1	0·8	2·4	10
35–	20	27	16	24	20	45	0·8	2·2	3·5	26
40–	19	34	14	32	47	105	0·8	3·3	11	52
45–	16	44	15	33	63	174	8	7	18	89
50–	24	50	18	45	104	288	11	13	40	138
55–	19	80	13	62	184	469	15	17	40	201
60–	22	123	19	81	252	719	28	19	65	287
65–	20	162	18	113	377	1033	32	24	80	384
70–	*16*	169	*12*	126	*386*	1570	*34*	32	*93*	379
75–	*3*	211	*19*	136	*426*	2432	*42*	33	*70*	445
80+	*21*	150	*0*	104	*305*	3667	*33*	39	*18*	362

countries having good registration and diagnostic arrangements. At ages after 70 certification of cancer as cause of death is probably less complete than in many countries of that class.

Comparison with England and Wales, and with Canada, shows that Japanese rates in middle life are high for cancer of the stomach, oesophagus and uterus, low for intestine, mouth and pharynx, breast and lung, and about the same for larynx. Despite the very low mortality from breast cancer, the total risk of dying from breast or uterine cancer between ages 30 and 60 is only slightly below that in England and Wales. Leukaemia rates are almost constant throughout life in Japan and do not show the increase after age 50 seen in English statistics.

It is suggested that a search for possible reasons for the low breast and high uterine cancer mortality, and for the low intestinal and high gastro-oesophageal cancer mortality in Japan might be helpful to the study of causation of these forms of cancer.

REFERENCES

Annual Epidemiological and Vital Statistics, 1952.—(1955) World Health Organisation, Geneva.

Mortality Trends in Canada.—(1955) National Cancer Institute of Canada, Toronto.

MAISIN, J. H. AND LANGEROCK, G.—(1955) *Schweiz. Z. allg. Path.*, **18**, No. 4, 690.

SEGI, M., FUKUSHIMA, I., FUJISAKU, S., HONMA, H., MIKAMI, Y., KURIHAMA, M. AND SAITO, S.—(1955) *Cancer Mortality Statistics in Japan* 1900–1954. Tohoku University, Sendai.

SEGI, M.—(1955) *Schweiz. Z. allg. Path.*, **18**, No. 4, 668.

STOCKS, P.—(1955) *Brit. J. Cancer*, **9**, 487.

Transactions of 5th Meeting of Internat. Society of Geographical Pathology.—(1955) *Schweiz. Z. allg. Path.* **18**, No. 4, 385–950.

WYNDER E. L., CORNFIELD, J., SCHROFF, P. D. AND DORAISWAMI, K. R.—(1954) *Amer. J. Obstet. Gynec.*, **68**, 1016.

THE GEOGRAPHICAL DISTRIBUTION OF CANCER OF THE STOMACH IN THE NETHERLANDS (PERIOD 1946–1952)

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Received for publication February 23, 1956

THE various problems in relation to the study of the causes of cancer of the stomach could be approached in two principally different ways :

(1) *Laboratory, genetic and clinical research*, in particular cytological, histological, biochemical and other related studies.

(2) *Medical-geographical research :* starting from the geographical distribution of cancer of the stomach in the Netherlands, the deeper causes of this distribution could be studied which possibly could give a clue to the cause of cancer of the stomach as such.

Sources of Information

As no morbidity data are available from all parts of the Netherlands the present study is based only on mortality data collected by the Netherlands Central Bureau of Statistics. In the present study only the period 1946–1952 was taken into consideration giving the best picture of the present stomach cancer mortality in the Netherlands. This period also has the advantage that in 1947 a population census took place which enabled us to calculate the mortality figures very accurately. For the seven years period 1946–1952 rates were obtained for the various 5-year age groups and classified according to sex, year and municipality.

The Netherlands Central Bureau of Statistics includes in the stomach cancer mortality figures : cancer of the stomach, cardia, pylori, papilla vateri, duodenum, sarcoma of the stomach, scirrhus. In view of the very small figures for most of these separate localisations and considering the uncertainty of diagnosis in many of these cases it was considered of greater value to combine these figures into one stomach-cancer-mortality figure.

Medical-geographical Methods Used

The mortality figures, classified according to sex and age groups for the various municipalities (smallest administrative units in the Netherlands), were presented in various municipal maps by using a special colouring system indicating various groups of stomach cancer mortality frequency (Fig. 1 and 2). It is possible that small differences between two neighbouring municipalities (particularly small ones) are only caused by differences in diagnosis or age group structure above 50 years of age. However, if a large group of municipalities, either bordering each other or being close together, shows considerably higher cancer frequency figures

than a similar large group of municipalities in another part of the province it is not very likely that only differences in diagnosis or age group structure can be considered to be the cause of the observed difference.

As indicated in Fig. 1 and 2 the geographical distribution of cancer mortality was not indicated for each 5-year age group separately, but the total mortality in the age groups 50 years and older was taken into consideration, because the municipal figures for the 5-year age groups are usually too small to be of any statistical significance. Five-year age group maps could be made only for the provincial distribution as indicated in Fig. 3, 4, 5 and 6. As differences in age group structure above the age of 50 could influence considerably the observed geographical distribution, apart from the maps based on the population above 50 years of age, similar maps were prepared on the basis of the population of 65 and older. For the age group 75 and older it was considered of no value to prepare such maps, as the observed differences have no statistical significance any more. The author realises that despite this age group correction still certain mistakes could occur in our maps. However, for our regional studies those possible errors have little importance for the following reasons.:

(1) As indicated in Fig. 1 and 2 the frequency groups lie rather far apart, ranging from 0–10 to 40 and over per 1000.

(2) Observed differences are only considered to be of any importance if the cancer frequencies in a group of neighbouring municipalities are more than 50 per cent above the average of the country.

Groups of municipalities with stomach cancer mortalities 50 per cent and more above the average of the country are called "PLUS AREAS", those 25 per cent and more below the average of the country are indicated as "MINUS AREAS".

Altogether six geographical distribution maps were prepared: two for the male population above 50 and above 65 years of age, two for the female population and two for the total population above 50 and 65 years of age. Only two of these maps are reproduced in this report (Fig. 1 and 2).

FIG. 1.—Total number of males, above the age of 50, who died from stomach cancer, per municipality, per 1000 male inhabitants above the age of 50. Average stomach cancer mortality in the Netherlands: 15·07 per 1000 males above the age of 50 years. (Based on data of the Central Bureau of Statistics in the Netherlands for the period 1946–1952.)

Notes: 1. "Stomach cancer mortality" comprises all deaths recorded by the Central Bureau of Statistics under the headings: cancer of the stomach, cardia, pylorus papilla vateri, duodenum, sarcoma of the stomach, scirrhus. 2. The municipalities Elten (Gelderland) and Tuddern (Limburg) were not included in our data because no data are available yet. 3. For all population data (number of inhabitants > 50 and > 65 years) the population census 1947 was used.

Scale = 1 : 400,000.

Group 1 = > 40	stomach cancer deaths (during 7 years) per 1000 males above the age of 50.	"Plus" areas.
„ 2 = 31–40	Ditto	
„ 3 = 21–30	„	
„ 4 = 16–20	„	
„ 5 = 11–15	„	"Minus" areas.
„ 6 = 0–10	„	

Fig.1

Possible Effect of Differences in Age Groups Structure Above 50 Years of Age

Despite our age group corrections (above 50 and above 65 years of age) still a number of physicians are inclined to believe that the greatest part of the observed geographical differences are only due to local differences in age group structure above 50 or 65. In a former publication (Diehl and Tromp, 1954) the various analyses made by the authors were summarized which contradict this assumption. If a large number of municipalities are classified according to their average stomach cancer frequency (on the basis of the population over 50) from high to low and the above mentioned assumption were true, we would expect a decrease of the percentages of population over 65 in these municipalities. Our studies clearly indicated that such a relation does not exist; in other words, a small part of the observed differences may be due to differences in age group structure above 65, but the latter cannot explain the main part of the observed differences.

Further support for this conclusion is given by the fact that the regional distribution observed in the maps based on the population over 50 is practically the same as the one in the maps based on the population over 65.

A second argument in favour of the statement above is the observation that in case of the provincial distribution maps (Fig. 3, 4, 5 and 6), which were made both for separate 5 year age groups after age 40 and for the total population of over 50 and 65 respectively, the same general distribution was observed. Further details of this study will be discussed later on in this report.

The Magnitude of the Mortality Figures

The data used in the various maps are based on 14,044 deaths (during the period of seven years) in the male population above 50 years of age and 10,021 deaths in the female population of the same age group. For the population above 65 these figures are respectively 10,063 and 7,597. The provincial distribution of these figures is as follows:

TABLE I.

	Province.									
	Gr.	F.	D.	O.	Ge.	U.	N.H.	Z.H.	Z.	N.B.	L.
Males > 50 years .	773	965	457	977	1372	681	2711	2835	685	1681	907
Females > 50 years .	526	642	278	634	1010	546	1907	2032	464	1212	770

FIG. 2.—Total number of females, above the age of 50, who died from stomach cancer, per municipality, per 1000 female inhabitants above the age of 50. Average stomach cancer mortality in the Netherlands: 10·09 per 1000 females above the age of 50 years. (Based on data of the Central Bureau of Statistics in the Netherlands for the period 1946–1952.)
See Notes 1, 2 and 3 for Fig. 1.
Scale = 1 : 400,000.

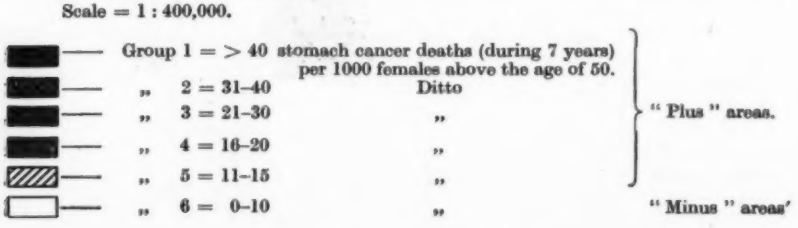
Group 1 = > 40 stomach cancer deaths (during 7 years) per 1000 females above the age of 50.
" 2 = 31–40 Ditto
" 3 = 21–30 "
" 4 = 16–20 "
" 5 = 11–15 "
" 6 = 0–10 "
" Plus " areas.
" Minus " areas

Fig. 2

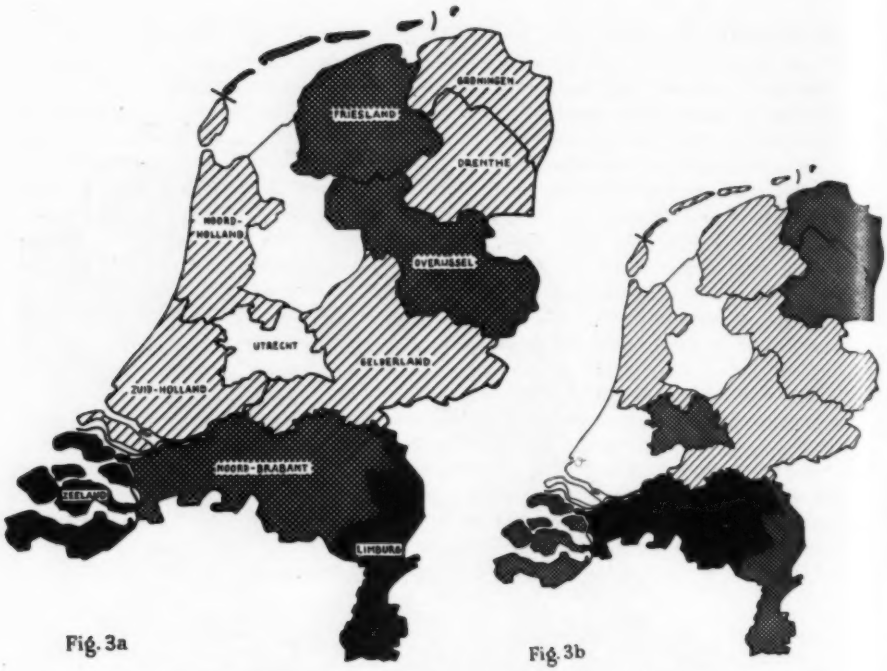

Fig. 3a

Fig. 3b

FIG. 3, 4, 5, 6.—Geographical distribution of stomach cancer mortality in the 11 provinces of the Netherlands in the male population (per 5-year age group, per 10,000 males of each age group) during the 7-year period 1946–1952. (Standardisation based on population census in 1947.) (Mortality data collected by the Central Bureau of Statistics of the Netherlands.)

Cancer mortality >25 per cent
" " 0–25 " " } Above the average for the country.

" " 0–25 " "
" " >25 " " } Below the average for the country.

FIG. 3a.—Age group 40–44. Total number of deaths in absolute figures : 278.
Total number of deaths per 10,000 males : 9·3.
" Plus " provinces { >25 per cent = 11·6. ; 0–25 " " = 9·3–11·6.
" Minus " provinces { 0–25 " " = 7·0–9·3. ; >25 " " = <7·0.

FIG. 3b.—Age group 45–49. Total number of deaths in absolute figures : 457.
Total number of deaths per 10,000 males : 18.
" Plus " provinces { >25 per cent = >22·5. ; 0–25 " " = 18–22·5.
" Minus " provinces { 0–25 " " = 13·5–18. ; >25 " " = <13·5.

Problem of Increase and Decrease of Stomach Cancer Mortality

During the period 1903–1930 the average yearly stomach cancer mortality in the Netherlands per 10,000 of the population (males and females together) over 40 was rather constant. It fluctuated between 22 and 24, with highest values in 1911 and 1913 (24·1). These mortality figures are based on the total cancer mortality of the stomach and liver together because in that period these two localisations were not separated. These figures are particularly untrustworthy if the cancer mortality is considered for each sex separately because liver cancer mortality in the female population is almost twice as high as in the case of the male population.

After 1930 when cancer mortality of the stomach and of the duodenum (without liver) was distinguished the average yearly rate was reduced to 15 per 10,000 of the population. During the period 1931–1949 the following decrease in stomach cancer mortality can be observed :

15·40 (in 1931)–15·76–15·10–14·58–14·65–14·81–14·63–14·67–14·01–14·36 (1940) –13·66–13·23–13·16–12·85–12·12 (1945)–10·82–11·32 11·31–11·98 (1949).

The observed decrease could be a true one, but may be partly due to various causes not related to cancer as such. For example it is possible that in the first years after 1930 a number of physicians did not separate sufficiently cancer of the liver and of the stomach in their death certificates. Future studies may reveal whether the observed decrease is a true one.

General Geographical Distribution of Cancer of the Stomach in the Netherlands

(A) *Geographical distribution based on the population over 50 and 65 years of Age:*

Male population.—Stomach cancer mortality of the male population is about 1·5 times as high as that of the female population. However, apart from this difference the following summary will indicate a number of similarities in the geographical distribution for the two sexes.

High stomach cancer mortality is observed in the provinces of Groningen, Friesland S.W. part of Drenthe, a N.S. strip in the middle part of Overijssel, the northern part of Noordholland (so-called Westfriesland), the eastern part of Noordbrabant, the northern part of Limburg and particularly high values in the province of Zeeland (300–600 per cent above the average of the country).

Low stomach cancer mortality is found particularly in the southern part of the province of Gelderland (so-called Betuwe), in the area north of the Rhine-Maas river district (in the provinces of Gelderland, Utrecht and Zuidholland) and in the southern part of the province of Noordholland.

In Table II the average stomach cancer mortality for the various provinces based on the male population over 50 and 65 years of age is indicated. For each of these provinces the percentages of municipalities were determined with average cancer mortality above the average of the country (so-called " + " municipalities) and below this average (so-called " — " municipalities).

Female population.—High stomach cancer mortality is found in the provinces of Groningen, Friesland S.W. part of Drenthe, N.S. strip in the middle part of Overijssel, the northern part of Noordholland, the eastern part of Noordbrabant, the province of Limburg and particularly high values in the province of Zeeland.

TABLE II.

	Province.											
	Z.	F.	D.	N.B.	Gr.	L.	O.	N.H.	Ge.	U.	Z.H.	Netherl.
Stomach cancer mortality per 1000 m. > 50 years	22·5	19·1	18·1	17·2	16·5	16·1	15·8	14·8	13·6	13·0	12·7	15·07
Percentage " + " munic. m. > 50 years	70	75	62	67	70	58	53	67	43	57	46	59
Stomach cancer mortality per 1000 m. > 65 years	43·2	35·7	36·6	33·0	31·8	31·8	30·6	32·6	26·6	27·1	27·5	30·83
Percentage " + " munic. m. > 65 years	65	64	59	51	59	39	38	68	34	55	46	51

Note : The figures above represent the average stomach cancer mortality during seven years. For mean annual figures the figures above must be divided by 7.

In other words, the same distribution is found as in the case of the male population, only in the province of Limburg the difference between the mortality frequency of males and females, is smaller than in the other provinces.

Low stomach cancer mortality frequencies are found in the same areas as in the case of the male population.

FIG. 4*a*.—Age group 50–54. Total number of deaths in absolute figures : 842.
Total number of deaths per 10,000 males : 35·5.
" Plus " provinces { >25 per cent = >53. / 0–25 " " = 35–53.
" Minus " provinces { 0–25 " " = 18–35. / >25 " " = <18.

FIG. 4*b*.—Age group 55–59. Total number of deaths in absolute figures : 1293.
Total number of deaths per 10,000 males : 64.
" Plus " provinces { >25 per cent = >80. / 0–25 " " = 64–80.
" Minus " provinces { 0–25 " " = 48–64. / >25 " " = <48.

FIG. 4*c*.—Age group 60–64. Total number of deaths in absolute figures : 1857.
Total number of deaths per 10,000 males : 109.
" Plus " provinces { >25 per cent = >136. / 0–25 " " = 109–136.
" Minus " provinces { 0–25 " " = 82–109. / >25 " " = <82.

FIG. 4*d*.—Age group 65–69. Total number of deaths in absolute figures : 2568.
Total number of deaths per 10,000 males : 188.
" Plus " provinces { >25 per cent = >235. / 0–25 " " = 188–235.
" Minus " provinces { 0–25 " " = 141–188. / >25 " " = <141.

Fig. 4a

Fig. 4b

Fig. 4c

Fig. 4d

TABLE III.

	Province.											
	Z.	L.	N.B.	F.	D.	Gr.	O.	Ge.	N.H.	U.	Z.H.	Netherl.
Stomach cancer mortality per 1000 f. > 50 years	14·8	13·6	12·5	12·4	11·5	10·5	10·4	9·8	9·3	9·0	8·1	10·09
Percentage " + " munic. f. > 50 years	65	69	64	73	53	59	49	45	53	52	40	55
Stomach cancer mortality per 1000 f. > 65 years	28·7	27·9	24·6	24·1	24·7	22·4	21·3	20·1	20·5	18·9	17·8	21·26
Stomach cancer mortality per 1000 m. + f. > 50 years	18·6	14·9	14·9	15·7	14·9	13·4	13·0	11·7	11·9	10·8	10·3	12·53

For mean annual figures the data above have to be divided by 7.

(B) *Geographical distribution based on 5-year age groups of the population above the age of* 40 (*Fig.* 3, 4, 5, *and* 6).

As stated above a five-year age group analysis is not possible for the municipalities separately, but could be carried out for the various provinces. In Tables IV and V a summary is given of the stomach cancer mortality in the eleven provinces

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FIG. 5*a*.—Age group 70–74. Total number of deaths in absolute figures : 2980.
Total number of deaths per 10,000 males : 304.
" Plus " provinces { >25 per cent = >380. / 0–25 " " = 304–380.
" Minus " provinces { 0–25 " " = 228–304. / >25 " " = <228.

FIG. 5*b*.—Age group 75–79. Total number of deaths in absolute figures : 2466.
Total number of deaths per 10,000 males : 443.
" Plus " provinces { >25 per cent = >553. / 0–25 " " = 443–553.
" Minus " provinces { 0–25 " " = 333–443. / >25 " " = <333.

FIG. 5*c*.—Age group 80–84. Total number of deaths in absolute figures : 1502.
Total number of deaths per 10,000 males : 567.
" Plus " provinces { >25 per cent = >709. / 0–25 " " = 567–709.
" Minus " provinces { 0–25 " " = 425–567. / >25 " " = <425.

FIG. 5*d*.—Age group 85 and older. Total number of deaths in absolute figures : 564.
Total number of deaths per 10,000 males : 625.
" Plus " provinces { >25 per cent = >781. / 0–25 " " = 625–781.
" Minus " provinces { 0–25 " " = 469–625. / >25 " " = <469.

Fig. 5a

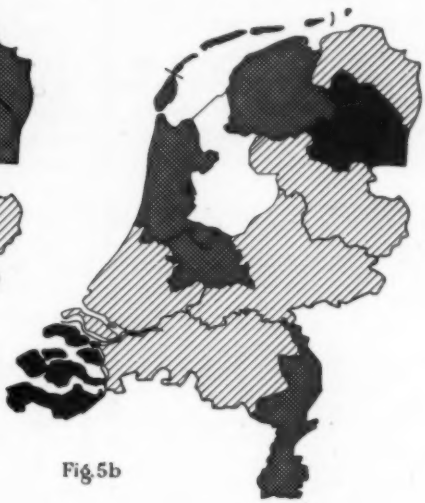

Fig. 5b

Fig. 5c

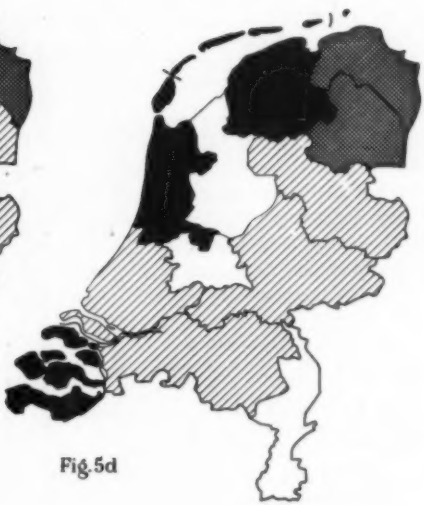

Fig. 5d

TABLE IV.—*Stomach Cancer Mortality in the Netherlands in the Male Population (per 10,000 males) in the Various Age Groups, during the Seven-year Period 1946–1952*

Prov.	Age-groups. Differences between provincial rates per 10,000 males and the corresponding national rate (as given in brackets). 40–44 (9·3)	45–49 (17·6)	50–54 (35·5)	55–59 (64·4)	60–64 (109·2)	65–69 (187·7)	70–74 (303·9)	75–79 (442·8)	80–84 (567·4)	85 e.o. (625·3)	Total "plus" prov. Total.	> 25%	Total "minus" prov. Total.	> 25%	Average population. (150·7) >50 yrs.	(308·8) >65 yrs.
Gr.	−0·3	+1·6	+6·8	+10·8	−7·6	−4·3	+20·6	−36·2	+14·14	+51·3	6	—	4	—	+15	+9
Fr.	+0·7	−2·1	+10·6	+10·3	+18·6	0	+64·7	+21·1	+27·9	+162·4	8	1	1	—	+41	+48
D.	−2·0	+3·1	−1·5	+22·5	+1·8	+52·9	+13·7	+112·4	+66·8	+97·2	7	3	3	—	+31	+58
O.	+1·3	−2·6	+6·2	+4·6	+10·1	+7·6	−7·0	−47·4	−7·7	−94·6	5	—	5	—	+8	−3
Ge.	−2·1	−1·3	−6·8	−11·6	−13·1	−30·5	−47·3	−37·9	−147·6	−49·4	0	—	10	1	−14	−42
U.	−3·8	+1·0	−2·7	−20·1	−17·0	−15·9	−53·9	+23·3	−147·6	−196·7	2	—	8	4	−20	−38
N.H.	−0·3	−0·3	−3·6	−2·1	+4·7	+23·7	+14·5	+15·8	+24·3	+225·1	6	1	4	—	−2	+8
Z.H.	−1·0	−4·1	−7·2	−5·9	−14·3	−14·1	−37·5	−32·6	−5·0	−150·8	0	—	10	1	−23	−34
Z.	+6·0	+4·7	+26·4	+14·5	+30·9	+70·2	+61·4	+128·5	+353·2	+255·8	10	7	0	—	+75	+123
N.B.	+0·2	+6·1	+12·0	+11·6	+14·4	+9·7	+45·7	−4·3	−7·4	−19·8	7	1	3	—	+22	+22
L.	+5·5	+2·3	+8·7	+13·1	+22·1	−16·8	+32·6	+38·8	+148·4	−274·4	8	2	2	1	+11	+9

TABLE V.—*Stomach Cancer Mortality in the Netherlands in the Female Population (per 10,000 Females) in the Various Age Groups, during the Seven-year Period 1946–1952*

Prov.	Age-groups. Differences between provincial rates per 10,000 females and the corresponding national rate (as given in brackets). 40–44 (5·2)	45–49 (11·4)	50–54 (20·9)	55–59 (36·8)	60–64 (67·4)	65–69 (114·0)	70–74 (195·9)	75–79 (304·7)	80–84 (418·5)	85 e.o. (523·4)	Total. "plus" prov. Total.	> 25%	Total. "minus" prov. Total.	> 25%	Average population. (100·9) >50 yrs.	(212·6) >65 yrs.
Gr.	+0·2	+3·3	−7·3	−5·6	−19·8	+2·1	−18·2	+34·9	+5·7	+5·4	6	1	4	2	+4	+11
Fr.	−1·0	−1·2	−5·5	+6·5	+12·5	−4·8	+19·3	+39·2	−9·8	+186·9	5	1	5	1	+23	+28
D.	0·0	0·0	−4·6	−14·8	+25·7	+18·9	+31·0	+43·6	+47·4	+15·5	6	—	2	1	+14	+34
O.	+0·1	−4·3	−0·3	+7·2	+7·0	+9·5	−10·0	+15·6	+18·2	−151·5	6	—	4	2	+3	0
Ge.	+0·4	+0·5	−0·9	−1·4	−10·0	−16·5	+2·1	−27·6	+5·5	−31·7	4	—	6	—	−3	−12
U.	−2·5	−0·4	+6·3	−7·4	−18·2	−17·4	−25·5	−35·1	+33·0	+86·7	2	—	8	2	−11	−24
N.H.	+1·3	−0·7	−1·5	−5·0	−7·0	−9·2	−12·1	−0·7	+16·4	+49·8	3	—	7	—	−8	−8
Z.H.	−1·0	+1·8	−4·8	−8·7	−15·8	−15·6	−34·8	−46·5	−62·3	−84·0	1	—	9	—	−20	−35
Z.	−0·6	−0·0	+4·4	+24·5	+30·5	+49·0	+50·0	+76·2	+125·4	+152·3	9	7	1	—	+47	+74
N.B.	+1·2	+2·3	+5·2	+15·6	+16·5	+33·5	+45·3	+29·5	+49·6	−37·4	9	3	1	—	+24	+33
L.	+1·9	+2·4	+131	+11·3	+50·8	+52·9	+99·1	+81·5	+49·1	+66·9	10	7	—	—	+35	+66

Note. + and − figures in age group columns indicate differences between provincial mortality and the average mortality of the country as a whole for that age group.

"Plus" provinces > 25 per cent indicates for each province how many times in the various age groups the average stomach cancer mortality is more than 25 per cent above the average of the country.

"Minus" provinces > 25 per cent indicates for each province how many times in the various age groups the average stomach cancer mortality is more than 25 per cent below the average for the country.

Average population > 50 years or > 65 years means average stomach cancer mortality per 10,000. Figures between brackets in age group columns indicate average stomach cancer mortality in these age groups for the country as a whole.

of the Netherlands for the male and female population separately, per 10,000 in 5-year age groups, during the seven year period 1946–1952. These tables permit us to draw the following conclusions :

Male population.—(1*a*) Zuidholland and Gelderland are characterized by a low average stomach cancer mortality for all the ten 5-year age groups separately.

(1*b*) Utrecht rates are low for 8 of the 10 5-year age groups and in 4 age groups more than 25 per cent below the average of the country.

(2*a*) The provinces of Zeeland and Friesland (except the age group 45–49) are characterized by high stomach cancer mortality, particularly Zeeland with stomach cancer mortality 25 per cent above the average of the country in 7 of the 10 age groups.

(2*b*) Also Drenthe, Noordbrabant and Limburg show a high stomach cancer mortality in 7–8 of the 10 age groups.

(2*c*) The relatively low stomach cancer mortality in the province Noordholland as a whole is only due to the very low values in the populous southern part of this province which counteracts the high rates in the northern part (so-called Westfriesland).

Female population.—A more or less similar distribution is found as in the case of the male population, i.e. low stomach cancer mortality in the provinces of Gelderland, Utrecht and Zuidholland and particularly high stomach cancer mortality in the provinces of Zeeland, Limburg and Noordbrabant.

For 6 of the 11 provinces stomach cancer mortality is represented graphically in Fig. 7. This picture again shows clearly that Zeeland is a province with high cancer mortality in all age groups, the province of Gelderland being low. The abnormal behaviour of the curves above the age of 75 is due to the small number of mortality cases in these highest age groups, causing erroneous standardized figures.

Ratio between Stomach Cancer Mortality in Male and Female Populations

Stomach cancer mortality in the male population is in general larger than in the female population. In Table VI we have indicated the ratio between the average provincial stomach cancer mortality per 1000 males above the age of 50 and per 1000 females above the age of 50. This ratio is rather constant, contrary to similar figures for lung cancer mortality in both sexes. The ratio for stomach cancer mortality fluctuates between 1·4 and 1·6 except in the province of Limburg.

TABLE VI.

	Province.											
	Z.	F.	D.	N.B.	Gr.	L.	O.	N.H.	Ge.	U.	Z.H.	Netherl.
Stomach cancer mortality per 1000 m. > 50 years	22·5	19·1	18·1	17·2	16·5	16·1	15·8	14·8	13·6	13·0	12·7	15·1
Ratio stomach cancer mortality m./f. above 50 years	1·5	1·5	1·6	1·4	1·6	1·2	1·5	1·6	1·4	1·4	1·6	1·5

Average population > 50 years or > 65 years means average stomach cancer mortality per 10,000. Figures between brackets in age group columns indicate average stomach cancer mortality in these age groups for the country as a whole.

Possible Relationship between Stomach Cancer Mortality and Size of Municipalities

In Table VII a summary is given of the average stomach cancer mortality in the various municipal groups based on the total population of these municipalities.

TABLE VII.

	Number of inhabitants per municipality.					
	< 5000	5,000–10,000	10,000–20,000	20,000–50,000	50,000–100,000	> 100,000
Average stomach cancer mortality per 1000 m. > 50 years	18·87	17·12	17·43	14·72	12·55	12·36
Same m. > 65 years . . .	35·88	32·88	33·89	30·01	26·41	27·00
Same f. > 50 years . . .	13·07	12·36	11·16	9·80	9·07	8·07
Same f. > 65 years . . .	25·95	25·14	23·26	20·63	19·18	17·52

Table VII clearly indicates a decrease in average stomach cancer mortality with increasing size of the municipality, an observation opposite to the lung cancer mortality which increases in the Netherlands with increasing size of the municipalities.

Possible Relationship between Stomach Cancer Mortality and Type of Municipality

The municipalities of the Netherlands can be divided in two large groups: typical rural municipalities with more than 50 per cent of the working population being engaged in agriculture; typical industrial municipalities with more than 40 per cent of the working population being engaged in industry. It is realised that this classification for the industrial municipalities is not quite satisfactory because not all of the typical industrial municipalities according to this classification belong to the typical "air polluting" industrial centres.

A statistical study indicates the following:

(1) Both for the male and female population in the Netherlands as a whole the average stomach cancer mortality in the typical rural municipalities is higher than in the industrial ones (which is opposite to the lung cancer mortality). Per 10,000 males over 50 years the stomach cancer mortality in typical agricultural and industrial municipalities is respectively: 190 and 136; for females 133 and 93.

(2) The same is true for the various provinces separately in the female population; in the male population it is true for all provinces except Groningen, Friesland and Drenthe with lowest stomach cancer mortality figures in the agricultural centres.

FIG. 6*a*.—Age group 50 and older. Total number of deaths in the Netherlands in the male population per 10,000 males of 50 and older: 151.

FIG. 6*b*.—Age group 65 and older. Total number of deaths in the Netherlands in the male population per 10,000 males of 65 and older: 309.

FIG. 6*c*.—Age group 50 and older. Total number of deaths in the Netherlands in the female population per 10,000 females of 50 and older: 100·9.

FIG. 6*d*.—Age group 65 and older. Total number of deaths in the Netherlands in the female population per 10,000 females of 65 and older: 212·6.

Fig.6a

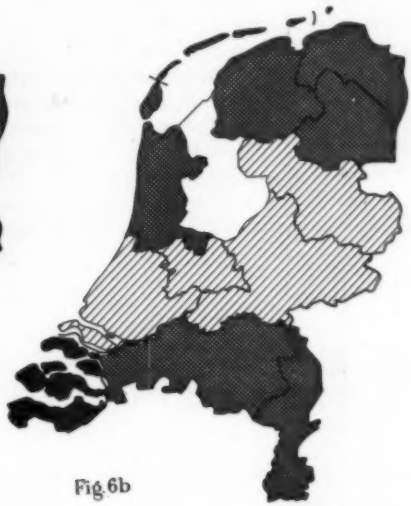

Fig.6b

Fig.6c

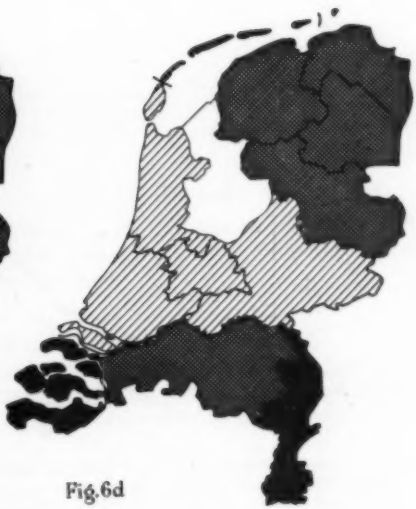

Fig.6d

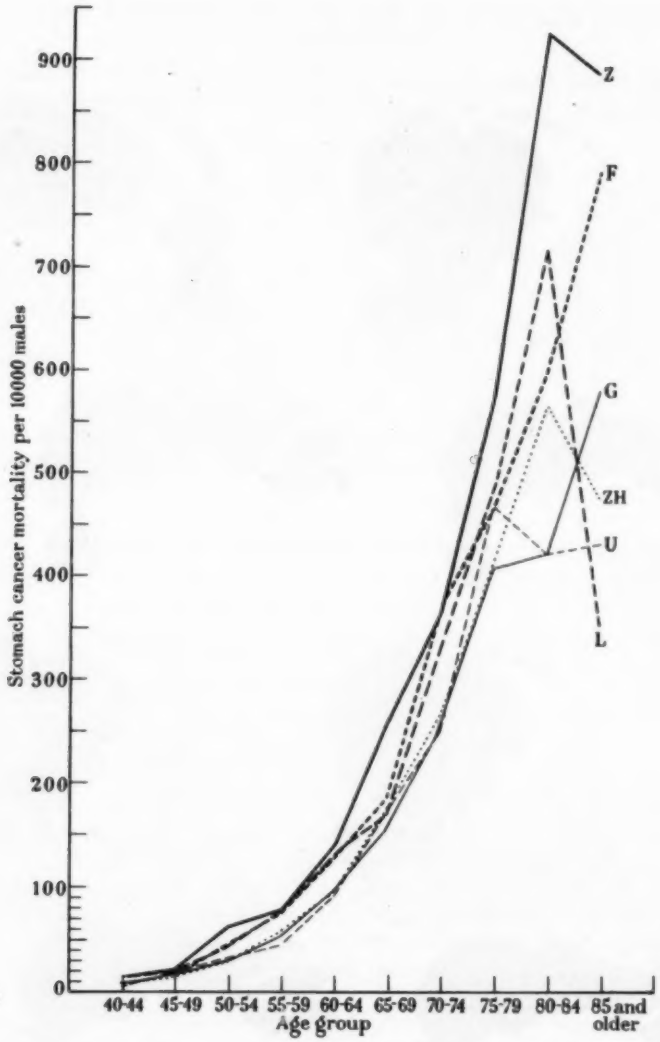

FIG. 7.—Graphic representation of stomach cancer mortality in 6 provinces of the Netherlands in the male population (per 5-year age group, per 10,000 males of each age group) during the 7-year period 1946–1952. Standardisation based on population census in 1947. Mortality data collected by the Netherlands Central Bureau of Statistics.

——— Zeeland
– – – – – Limburg
. . . . . Friesland
——— Gelderland
– – – – – Utrecht
. . . . . Zuid-Holland

Possible Relationship between Stomach Cancer Mortality, Population Density and Average Income

If the provinces are classified according to their average stomach cancer mortality from high to low no clear relationship can be observed with average population density and average annual income. However, it is worth mentioning that in the five provinces with highest stomach cancer mortality (Zeeland, Friesland, Drenthe, Noordbrabant and Groningen) the average population density is below the average of the country, whereas of the other six provinces, in four the population density is above the average of the country. A similar weak relationship is found for the average income, i.e. lowest average income in provinces with highest cancer mortality and vice versa, a relationship opposite to lung cancer mortality, which shows a low mortality in provinces with low average income.

TABLE VIII.

	Province.											
	Z.	F.	D.	N.B.	Gr.	L.	O.	N.H.	Ge.	U.	Z.H.	Netherl.
Stomach cancer mortality per 1000 m. > 50 years	22·5	19·1	18·1	17·2	16·5	16·1	15·8	14·8	13·6	13·0	12·7	15·07
Average population density	161	145	109	258	206	339	210	712	220	441	862	315
Average annual income per inh.in guilders (1946)	796	724	595	692	796	707	691	980	709	854	924	817

Note.—Population density is the average number of inhabitants per square kilometre. This is not correct for a province like Friesland, with very large municipalities (in square kilometres), with a number of densely populated living centres, but the average density per square kilometre being small.

Possible Relationship between Stomach Cancer Mortality and Soil

This relationship was described extensively by Tromp and Diehl (1955). A mathematically significant correlation was found between certain types of soil in the Netherlands and stomach cancer mortality.

REFERENCES

DIEHL, J. C. AND TROMP, S. W.—(1954) Statistical Analysis of the possible influence of differences in age group structure (above the age of 50) on the geographical distribution of Cancer (annex 2 to Vol. I, *Found. for the Study of Psycho-Physics* 1954).

TROMP S. W. AND DIEHL, J. C.—(1955) *Brit. J. Cancer.* **9**, 349.

THE SIGNIFICANCE OF SELECTION IN PROSPECTIVE INVESTIGATIONS INTO AN ASSOCIATION BETWEEN SMOKING AND LUNG CANCER

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Received for publication March 6, 1956

It will never be possible to furnish mathematical proof of a causal relationship between smoking and lung cancer. This means that, before action against smoking can be taken, in view of the danger of lung cancer, we must be satisfied on the following two points. First there must exist strong indications that smoking is an important causative factor in lung cancer, and, secondly, it must be highly improbable that one or more other factors could be the main cause.

As far as the latter point is concerned: all attempts to exculpate smoking have so far been unsuccessful. The longer such success remains unachieved, the smaller the probability that the greatest danger might lie in other still unknown factors.

As regards the first point: there is no doubt whatsoever that the indications that there is a danger inherent in smoking are very strong. The results of all retrospective investigations invariably implicate smoking.

However, against this retrospective method of investigation a serious objection can be raised: the material for comparison, a group of persons not suffering from lung cancer, is necessarily highly selected. In view of this, investigations by means of the so-called prospective method, against which, it was thought, this objection could not be raised, were set up. The preliminary results of two of these prospective investigations have now become known (Doll and Hill, 1954; Hammond and Horn, 1954). They seem fully to corroborate the conclusions reached previously in the retrospective investigations.

However, Berkson (1955) alleges that the prospective method also involves the danger of selection. Berkson attempts to demonstrate that this is the case in Hammond and Horn's material. He bases his opinion mainly on two facts: (1) the death rates found by Hammond and Horn are lower than the rates given by the official American mortality statistics, and (2) the percentage of non-smokers in Hammond and Horn's population exceeds the percentage found in the general American population.*

Berkson (1955) goes so far as to infer that the excess of the death rates for smokers, as found by Hammond and Horn (1954) in lung cancer and in coronary disease, might wholly be explained by the action of this selection. It seemed necessary, therefore, to test the correctness of this view thoroughly. This will be done in the present paper.

* Hammond and Horn's population was 187,766 American white males between the ages of 50 and 69. Wherever mention of "population" is made in our paper, it will mean these white males exclusively.

I.—*Selection as a Consequence of the Elimination from the Reference Population of a Number of Persons who at the Start of the Investigation were Seriously Ill.*

One glance at Table I seems to justify Berkson's (1955) first remark. Hammond and Horn's (1954) death rates for all causes are almost one-third lower than those of the official mortality statistics. Quite irrespective of smoking or non-smoking this material bears the mark of selection in favour of low death rates.

There are several reasons, however, why comparison of these death rates should not be made without comment. Hammond and Horn's males originated from only nine States. It is open to question, therefore, whether their reference population can be considered as representative of the entire American population, an assertion which, it must be admitted, was not made by them.

The majority of Hammond and Horn's population were exposed to risk during 12 low death rate summer-months and only 6 high death rate winter-months, which tended to make their over-all death rate lower than comparable figures based on exposure to risk during a single, complete calendar year.

Add to this that their death rates are computed from the $\frac{12}{20}$th part of all deaths occurring during the 20 months from March 1952 to October 1953 compared with the number of persons under investigation in March 1952, so that at the moment of the review the age of these persons was not 50–69 but $50\frac{1}{2}$–$69\frac{1}{2}$ approximately. Therefore, their death rates cannot really be compared with the official death rates for the 50–69 age group of the entire American population–which age group is approximately 7 months younger and has correspondingly lower death rates—as is done by Berkson (1955) in his Tables 4 and 7, and by us in our Table I.

TABLE I.—*Death Rates from All Causes per* 100,000 *White Males Aged* 50–69, *U.S.A.*

Age. 1.	Figures from official mortality statistics, 1952. 2.	Figures found by Hammond and Horn. 3.
50–54	1206	837
55–59	1891	1345
60–64	2793	1925
65–69	4089	2905

However, notwithstanding these objections, in order to furnish ourselves with a kind of standard by which to measure the importance of this selection, we will start, in what follows, from the assumption that Hammond and Horn's reference population does lend itself for such comparison.*

As Berkson remarks and as was pointed out by Hammond and Horn before, a number of persons, who at the moment of the interrogation were already seriously ill from the disease to which they would soon succumb, will not have been asked for co-operation or, if so, will have refused. It seems reasonable to suppose that these persons formed the major part of the "deficit" of deaths. Since, as far as we can see and as Berkson believes, this kind of selection is not in any way related to smoking, it will hardly have contributed to the excess of the death rates for smokers, as found by Hammond and Horn.

* See, for the computation of the "deficit" of deaths in Hammond and Horn's investigation, as compared with the official mortality statistics: the Appendix at the end of the present paper.

II.—*Selection as a Consequence of the Tendency of Many Smokers Not to Enter the Investigation*

To what degree the non-smokers are over-represented in Hammond and Horn's (1954) material cannot exactly be ascertained, one of the reasons being that the persons under review are not truly representative of the entire American population. However, Berkson's (1955) remark on page 329 and his Table 3 illustrate that he may be correct when he writes that the surplus of non-smokers must be rather big.

The reason for this surplus is most likely the psychological reluctance of many smokers to co-operate in an investigation which might prove the noxiousness of smoking, whereas, in contrast with this attitude, nearly all non-smokers—many of them perhaps from a propagandist point of view—are ready for co-operation.

The question arises to what extent, if there were no relation between smoking and death rates, the excess of the death rates for smokers as found by Hammond and Horn, could be considered a statistical consequence of this selection. It is clear that this question should be tackled quantitatively.

In this paper the computations necessary for this purpose will be made for the age group 50–54, after which the findings for the other age groups will follow in tabular form.

Our research is made with the help of a " model ", such as, in principle, is given in Berkson's Table I. The most important change we made is that, as far as possible, Berkson's rather arbitrarily chosen figures were substituted by the figures found by Hammond and Horn and by those of the official mortality statistics.

We start tentatively from the assumption that smoking is not related to death rates.

Berkson divides the selected population (selected by the elimination of a number of persons who at the moment of the interview were seriously ill, see Section I) into two groups.

Group 1 embraces those who, though also seriously ill at the moment of the interrogation, were prepared to co-operate. Berkson assumes that the number of these persons equals the number of those who primarily were eliminated on account of serious illness (see Appendix). The significance, for our research, of these arbitrary assumptions will be discussed later.

Group II includes those who at the moment of the interview were in good health, or at least not seriously ill.

This division is given schematically in our Table II.

TABLE II.—*Elimination of Seriously Ill Persons from Hammond and Horn's (1954), Reference Population, Age Group 50–54.*

Reference population.				
	Selected population of Hammond and Horn.			
1.	2.	3.	4.	5.
Eliminated.	Group I.	Group II.	Total I + II.	Total.
374	374	99,626	100,000	100,374

Berkson assumes that 80 per cent of the reference population are smokers. Starting from this assumption, we have subdivided the persons of Table II into smokers and non-smokers. This is done in Table III, in which the eliminated persons are no longer mentioned.

TABLE III.—*Hammond and Horn's* (1954) *Age Group* 50–54 *After First Selection has been Made, Assuming that* 80 *Per Cent of all Persons are Smokers.*

1. Smoker.	2. Group I.	3. Group II.	4. Total.
No	75	19,925	20,000
Yes	299	79,701	80,000
Total	374	99,626	100,000

The next step leads to a second selection which, contrary to the selection dealt with in Section I, definitely is related to smoking. Berkson assumes that 99 per cent of the non-smokers and 65 per cent of the smokers of Group II were prepared to co-operate. This means that 19,726 of the 19,925 non-smokers and 51,806 of the 79,701 smokers who were asked for co-operation, consented. As the totals for Groups I and II combined must take up the 100,000, the total for Group II must be 99,626. The deficit of persons in Group II, caused by this selection, must therefore be made good by means of interviewing more persons, of whom again 4 out of every 5 will be smokers. This replacement must be made proportionately to the above given figures for non-smokers and smokers. The result of this conversion is given in Column 3 of Table IV, the left part of which gives the figures for the persons under review.

TABLE IV.—*Computation of Death Rates from All Causes in Hammond and Horn's* (1954) *Age Group* 50–54 *After Second Selection had been Made, accepting certain assumptions as being correct.*

	Exposed.			Deaths.			
1. Smoker.	2. I.	3. II.	4. Total.	5. I.	6. II.	7. Total.	8. Death rate per cent.
No	75	27,474	27,549	74	129	203	0·737
Yes	299	72,152	72,451	296	338	634	0·875
Total	374	99,626	100,000	370	467	837	0·837

The seriously ill persons of Group I were assumed to have all co-operated, quite a reasonable supposition. Their distribution over non-smokers and smokers therefore remains one to four.

As a consequence of this second selection the percentage of non-smokers in our Table IV has increased from 20 to 27·5 per cent.

In the right half of Table IV the figures for deaths from all causes per 100,000, divided according to group and category, are given.

According to Berkson's (1955) assumption that 99 per cent of those belonging to Group I succumbed during the period under review (in this case 20 months), the total number of deaths in this group was 370. These 370 are

distributed over non-smokers and smokers in the ratio 1 to 4. Per 100,000 non-smokers and smokers combined Hammond and Horn (1954) found 837 deaths ; the figure for total deaths in Group II is therefore 837 — 370 = 467. According to our assumption that smoking is not related to death rates, these 467 deaths are distributed over non-smokers and smokers proportionately to the number of persons of both categories (Column 3, Table IV). An addition gives the figures for total deaths among non-smokers and smokers separately.

In Column 8 the rates for deaths from all causes are to be found. Using the death rate for non-smokers as the base, an increase of 19 per cent in the death rate for smokers is found. As we started from the assumption that smoking and death rates are not related, this 19 per cent increase, the consequence of the second kind of selection, is a pure artefact.

For the computation of the death rates for each particular cause of death* the left part of our Table IV can be used ; for the computation of the eliminated persons, see Appendix.

In Column 3 of Table V the percentages of the spurious excess of the death rates for death from different causes in smokers aged 50–54 are given.

If we consider what lies behind this spurious association between smoking and death rates, the following becomes evident. Suppose that, at the start of the investigation, no persons would have been so ill as to be classified in Group I. In that case, the non-cooperation of a certain percentage of smokers would result in the number of deaths occurring among them decreasing by the same percentage, and the death rates would remain unaltered. The same would apply to the non-smokers. Therefore, no spurious increase of death rates for smokers would be found. It is mainly the size of the figure for deaths in Group I, as compared with the figure for deaths in Groups I and II combined, which determines the size of this spurious increase.

In Table V the causes of death are arranged in order of decreasing size of the figures for primarily eliminated deaths (i.e. eliminated by the first selection), expressed as percentage of the number of deaths found by Hammond and Horn (i.e. all deaths of Groups I and II combined).

As the deaths of Group I occurred among the seriously ill persons who at the moment of the interview were willing to co-operate, and the eliminated deaths among those who were not, there must exist a close parallelism between the size of their figures for each cause of death. Therefore, the order of the numbers of deaths in Group I and of the numbers of the eliminated deaths, both expressed as percentage of the number of deaths found by Hammond and Horn, must be the same.

From Columns 2 and 3 of Table V it appears that the order of the percentages for the eliminated deaths—and therefore also for the deaths in Group I—and the order of the percentages for the spurious excess of the death rate for smokers is also the same. This seems to support the view expressed above as to the significance of the relative sizes of the figures for deaths in Groups I and II, for the size of the spurious excess in death rates for smokers.

* " All cancers " means : numbers 140–205, and " coronary disease " : number 420 of the detailed international classification of deaths, revision of 1948. " Other cancers " means : all cancers exclusive of lung cancer ; " other diseases " : all diseases exclusive of all cancers and of coronary disease. The figures for " lung cancer " are borrowed from Table 4 of Berkson, who obtained the figures from Mr. I. M. Moriyama.

TABLE V.—*Comparison of Percentages for Eliminated Deaths with Percentages for Spurious Excess of Death Rates in Smokers, Age 50–54.*

Cause of death. 1.	Eliminated deaths, expressed as percentage of deaths found by Hammond and Horn. 2.	Percentage of spurious excess of death rates in smokers. 65% of smokers co-operating. 3.	Percentage of spurious excess of death rates in smokers. 35% of smokers co-operating. 4.
"Other diseases"	43	34	102
Lung cancer	36	24	69
All cancers	34	22	59
"Other cancers"	33	22	58
All causes	31	19	50
Coronary disease	13	6	14

In coronary disease the excessively low percentage of the spurious increase of death rates for smokers corresponds with an excessively low percentage of eliminated persons. Unless coronary disease was associated with particularly little resistance to co-operation, this low percentage of eliminated persons would point to the fact that an unusually high percentage of victims to this disease falls among the persons belonging to Group II, that means among those who at the moment of the interview imagined themselves to be of good health. The result of Hammond and Horn's investigation therefore corroborates a fact, well known by clinical experience, that in general the duration of illness is much shorter in coronary disease than in most forms of cancer. On the other hand, duration of illness in "other diseases" —an exceptionally heterogenous group comprising chronic tuberculosis as well as accidents—appears to be much longer than in coronary disease.

As the spurious increase of death rates for smokers grows in proportion to the number of deaths in Group I compared with this number in Groups I and II combined, it follows that this increase would reach its maximum if all deaths occurred in Group I. In that case a spurious increase of the death rates for smokers of 52 per cent would be found for each separate cause of death. If not 65 per cent but only 35 per cent of the smokers were prepared to enter the investigation, percentages of spurious increases as mentioned in Column 4 of Table V would be found. These data are given to obtain a better insight into the real significance of our assumptions.

Table VI gives the following data for the different causes of death and for age groups: (1) (bold type) the percentages of excess of death rates for smokers, found by Hammond and Horn; (2) (in italics) the percentages of the spurious excess of death rates for smokers, computed in the present paper on the assumptions that smoking is not causally related to death rates, and that 65 per cent of the smokers who were in good health at the moment of the interview were prepared to answer the questionnaire.

In the different age groups great differences for the same cause of death exist. This will be shown to be partly due to the relatively small figures, partly to the fact that the meaning of a cause of death may differ from one age group to another. In the three lowest age groups the figures for deaths from lung cancer are so small that little significance should be attributed to the percentages of increase; the general trend of these percentages is clear, however.

TABLE VI.—*Percentage Excess of Death Rates for Regular Cigarette Smokers as Compared with Death Rates for Persons who Never Smoked Cigarettes Regularly.*

1. Age.	2.	3. All causes. %	4. All cancers. %	5. Lung cancer. %	6. "Other cancers." %	7. Coronary disease. %	8. "Other diseases." %
50–54	Figures found by Hammond and Horn	59	54	(676)	13	92	33
	Figures computed in present paper	*19*	*22*	*24*	*22*	*6*	*34*
55–59	Figures found by Hammond and Horn	41	59	(367)	21	65	14
	Figures computed in present paper	*17*	*19*	*17*	*20*	*6*	*34*
60–64	Figures found by Hammond and Horn	64	103	(1647)	63	85	31
	Figures computed in present paper	*20*	*21*	*52*	*15*	*8*	*36*
65–69	Figures found by Hammond and Horn	22	56	180	40	29	4
	Figures computed in present paper	*18*	*22*	*28*	*20*	*6*	*30*

In lung cancer and coronary disease Hammond and Horn's increases of death rates for smokers far exceed the spurious increases computed by us. Inasmuch as, at the start of the investigation, most non-co-operating smokers were in good health or at least not seriously ill, and most of them could have had no idea of what disease they would fall victim to later, there is no reason why the future lung-cancer patients would have felt less inclined to co-operate than those who were to die from other diseases. Therefore, a " strengthening " of our assumptions with the object of lifting our percentages for lung cancer to the level of those found by Hammond and Horn, would also cause proportional increases in the percentages for all other causes of death (Table V). In the group of " other cancers " our own percentages for the spurious increase would then exceed Hammond and Horn's percentages so phenomenally that we should wonder why smoking should give such good protection from all cancers with the exception of lung cancer.

Our conclusion therefore must be that the explanation for the excess of death rates for smokers in lung cancer and in coronary disease as found by Hammond and Horn, may only for a small part lie in the reluctance of smokers to co-operate whereas the greater part of this increase must be due to one or more other factors.

A closer examination of Table VI reveals the following: With *lung cancer* a greatly increased death rate for smokers exists, which cannot be explained by the above-mentioned way of selection. This increased death rate from lung cancer—which disease accounts for 20 per cent of all deaths from all forms of cancer in persons aged 50–69—pushes up the death rate for " *all cancers* ", and Hammond and Horn's percentage of increase therefore considerably exceeds the percentage for the spurious increase as computed in the present paper. With " other cancers " this is not so, or only to a much smaller degree. But, whereas the importance of lung cancer compared with other forms of cancer greatly diminishes with advancing age, the importance of other forms of cancer, such as cancer of the larynx and the mouth, with which some kind of association with smoking may be suspected, greatly increases. This might explain part of the

reversal in the percentages of increase in Column 6 of Table VI. Besides, we should not forget that in our highest age groups smoking habits differ a great deal from those of younger people.

Also with *coronary disease* Hammond and Horn found a considerable excess of the death rate for smokers. It should be stressed here that to conclude that there is a causal relationship between smoking and coronary disease, merely on the ground of this association, would be unwise. The same thing certainly applies to lung cancer. With lung cancer, however, there are a great number of facts implying causality ; with coronary disease this is not so or is so to a much smaller degree.

In " all causes " Hammond and Horn found a moderate increase. This is partly caused by two of the diseases included therein : lung cancer which shows a considerable excess but which amounts to only 3½ per cent of all deaths, and coronary disease which shows a smaller excess but amounts to no less than 36 per cent of all deaths.

There remains, still, " other diseases " in which, without exception, our figures exceed those found by Hammond and Horn. This may indicate that our assumptions are a bit " overweighted ": that the percentage of co-operating smokers was higher than 65 per cent.

Berkson's and our assumption that the psychological reluctance of smokers to co-operate in this kind of investigation may cause a spurious increase in death rate for each separate cause of death, seems well founded. It is clear that the more heavily a person smokes, the greater will be this psychological reluctance as well as the spurious increase in the death rate caused by it. Therefore, that the association of deaths, from diseases other than lung cancer, with cigarette smoking is also shown in Hammond and Horn's study in a positive correlation between the death rate and the amount of smoking, does not astonish us at all. On the contrary, it is just what we expected. It confirms our opinion that this material does indeed bear the sign of selection by the elimination of many smokers, and it does not evidence in any way against the great probability of the importance of smoking as a causal factor in lung cancer—and perhaps in coronary disease.

Just as in this investigation, the death rates in Doll and Hill's prospective investigation are lower than the rates given by the official mortality statistics. The reason for this was believed to be the same as is discussed in our Section I.

In contrast with the findings of Hammond and Horn (1954), Doll and Hill (1954) found no excess of death rates for death from " other diseases " and from " cancer other than lung cancer ", and only a small excess for death from " coronary disease ". This seems to indicate that among their smokers only little reluctance to co-operate existed, which would be in keeping with the fact that the percentage of smokers in their study, and the percentage of smokers found in a social survey of Greater London, are almost the same.

The findings in Doll and Hill's investigation, therefore, seem to support the view on the significance of this selection in prospective studies on smoking and lung cancer, as expressed in this paper.

SUMMARY AND CONCLUSIONS

Berkson (1955) points out, quite rightly, that, inasmuch as the death rates found by Hammond and Horn (1954) are lower than the death rates of the official

mortality statistics, their material should be considered as " selected " with respect to the entire American population.

Berkson also points out, quite rightly, that, in research into an association between smoking and lung cancer, due attention should be given to the psychological reluctance of smokers to co-operate. By means of a " model ", based on a number of assumptions, he proves that this reluctance may lead to distortion of the result of investigations like Hammond and Horn's.

Starting from similar assumptions and using a similar model, it appeared possible to us, to fix, approximately, the size of this distortion. As a result of this investigation we found :

(1) that because of the action of this selection, as caused by the tendency for many smokers to eliminate themselves, only a small part of the excess in death rates for lung cancer and for coronary disease with smokers can be explained as being spurious ; therefore, the major part of this excess is not due to this selection but to one or more other fractors ;

(2) that this selection gives a plausible explanation for the excess in death rates for smokers for " other diseases " and " other cancers ", so that the excess in death rates in these diseases should be considered as being wholly artificial ;

(3) that the spurious excess in death rates for smokers, as caused by this selection, is proportional to the quantity of cigarettes smoked ;

(4) that, when in the reference population the percentage of smokers is assumed as being 80, this selection causes a decrease of this percentage to 72·5, so that part of the " shortage " of smokers, found in Hammond and Horn's investigation, can be explained by the action of this selection.

If our opinion is correct, it follows that Hammond and Horn's death rates entitle us to believe in a large and true excess of death rates with smoking in lung cancer and in coronary disease, whereas the excess they recorded in " other cancers " and in " other diseases " should be regarded as spurious.

To conclude :

Two objections which are often raised against Hammond and Horn's results : (1) the excess in death rates among smokers from causes of death with which an association with smoking would seem very improbable, and (2) the small percentage of cigarette smokers, may quite plausibly be explained by the psychological reluctance of smokers to enter this kind of investigation.

If we accept this psychological reluctance as a fact, Berkson's suggestion does not undermine—on the contrary it supports—our belief that cigarette smoking is causally related to lung cancer, and perhaps to coronary disease.

APPENDIX

If we assume that, as regards death rates, Hammond and Horn's (1954) reference population is representative of the entire population of the U.S.A. their deficit of deaths from all causes in those aged 50–54 does not equal the difference between 1206 and 837, i.e. 369 (Table I). The reason is that Hammond and Horn's 100,000 persons were a selected group, owing to the elimination from the reference group of an unknown number of seriously ill persons—which elimination was responsible for the deficit of deaths.

We can make the following assumptions : (1) all of those who were eliminated died in the period under review—in this case during the next 20 months ; (2)

only half of these persons died during this period. The number of these persons will actually lie somewhere between both possibilities; probably nearer to the former than to the latter.

Let us first base our calculations on the former assumption. Suppose that, in order to reach their 837 deaths per 100,000 (Table I), Hammond and Horn had unknowingly eliminated X deaths. This being so, their reference population would have numbered $100{,}000 + X$. The number of deaths they should have found in these $100{,}000 + X$ persons would be:

$$\left(100{,}000 \times \frac{837}{100{,}000}\right) + X$$

According to the official statistics the number of deaths in these $100{,}000 + X$ were:

$$(100{,}000 + X) \times \frac{1206}{100{,}000}$$

As in both cases the number of deaths is the same, we find: $X = 374$.

If we base our calculations on the second assumption, we find: $X = 378$. The difference between these two figures is minimal. We have based our calculations on the first assumption, from which it follows that, as regards death rates, the reference population from which each 100,000 of Hammond and Horn's age group 50–54 were selected, consisted of 100,374 persons.

For the separate causes of death the deficit in those aged 50–54 can be computed with the help of the following formula:

$$\text{deficit} = \frac{P \times 100{,}374}{100{,}000} - Q$$

in which P means the official death rate per 100,000, and Q the death rate per 100,000 as found by Hammond and Horn.

REFERENCES

BERKSON, J.—(1955) *Proc. Mayo Clin.*, **30**, 319.
DOLL, R. AND HILL, A. B.—(1954) *Brit. med. J.*, i, 1451.
HAMMOND, E. C. AND HORN, D.—(1954) *J. Amer. med. Ass.*, **155**, 1316.
U.S. Dept. of Health.—(1954) Vital Statistics-Special Reports: National Summarie **40**, 57.

THE EPIDEMIOLOGY OF GASTRIC CANCER IN OSLO: MATERIAL, DIAGNOSIS AND MOBILITY OF POPULATION. SOME SOURCES OF ERROR

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Received for publication March 15, 1956

THE field of epidemiology, or endemiology, of cancer may be broadly defined as comprising knowledge of variations in distribution and behaviour of cancer among various ethnological groups within different localities in relation to any relevant local factors. Such knowledge is sought in order to elucidate the causes of cancer or to suggest experimental studies which may do so, with the ultimate aim of assisting in the prevention of cancer in man (Delafresnaye, 1950).

The difficulties inherent in planning and evaluation of statistics of this sort have been repeatedly stressed in the literature (Delafresnaye, 1950, Expert Committee on Health Statistics, 1952; Dunham and Dorn, 1955; Dorn and Cutler, 1955). On the basis of these and other considerations, the validity of some of the reported statistics on cancer epidemiology may be questioned. On the basis of a material of gastric cancer collected with the intention to study the possible role played by certain socio-economic factors this article aims to study how far some common sources of error can be avoided or evaluated under the rather favourable conditions present in Norway for such investigations.

MATERIAL

The population of Oslo offers several advantages for the study of a possible association between gastric cancer and socio-economic or other factors which may related to the etiology of the disease. The population is very homogeneous with regard to race, it is well registered, and the physio-geographic environment is rather uniform. The inhabitants of the capital who amount to some 440,000 persons seem to share the high mortality from gastric cancer presented by the Scandinavians in general. In Norway, 1952, the mortality rate for this disease per 100,000 was 55·2 and 37·7 for males and females, and the corresponding rate for Oslo was 48 and 37. The city comprises a so-called Inner Zone or " city proper " and an Outer Zone, the latter being incorporated in the city from the surrounding districts in 1948. The Inner Zone, which forms the basis for the main part of this investigation, is a relatively small city area covering some 18 square kilometres (some 7 square miles). The greater part of the population lives within this zone (in 1950: 281,000 inhabitants).

In several publications comparisons have been made between the occurrence (incidence, prevalence or mortality) of cancer in rather large municipal districts or other areas differing with regard to average socio-economic conditions. Since considerable variations in this respect may exist within such areas, minor entities may be constructed in order to obtain areas of a more homogeneous nature. Thus,

while formerly a subdivision of the Inner Zone into the 16 parishes has been used for various medical investigations, the present work is based on a primary subdivision of this zone into 209 "census tracts" elaborated by the Oslo Town Planning Office, in conformity with generally accepted criteria for homogeneity of urban areas (Schmid, 1938). In order to obtain sufficient data for statistical analysis, neighbouring areas showing very nearly the same mortality from gastric cancer have subsequently been grouped together.

A socio-economic analysis is further facilitated by the use of data obtained from socio-graphic investigations carried out in later years (Petersen, 1954). The city shows an internal social differentiation, with a rather sharp division between the eastern and the western half. This may to some extent be illustrated by the fact that the main residences of manual workers (e.g. when given in per cent of all male tax-payers) are situated within the eastern half of the city. Thus, in large areas of the eastern half, more than 70 per cent of the adult male population are manual workers, while the corresponding figure for the typical western zone is less than 30. This is partially explained by the distribution of industry which is mainly situated in the eastern zone and, to a certain extent, also near the centre of the city and in a few smaller areas in the western zone.

The present report is based on a review of all death certificates from Oslo during the five year period 1949–1953 in which the diagnosis of gastric cancer has been made. The total number recorded in this period was 851. In eight cases no address could be given, and these patients were discarded, leaving 843. The distribution of these by age and sex, is given in Table I. As will be seen, relatively few (0·4–0·3 per 10,000 per year) deaths occur below 50 years of age. On the other hand, there are reasons to believe that the diagnoses in patients aged 80 or more are somewhat less reliable than in the other age groups, as will be commented on later. Further investigations were therefore restricted to patients aged 50–79 (Table I)

TABLE I.—*Deaths from Gastric Cancer in Oslo, 1949–1953, by Age and Sex*

		Age.					
		–49.	50–59.	60–69.	70–79.	80–.	50–79.
Number of deaths	M.	31	79	138	146	76	363
	F.	22	54	79	136	82	269
Population December 1, 1950	M.	146,594	27,512	14,798	7,831	1,954	50,141
	F.	164,309	33,793	20,588	12,613	4,055	66,994
Dead per 10,000 per year	M.	0·4	5·7	18·6	37·3	77·6	14·4
	F.	0·3	2·9	7·7	21·6	40·4	8·0

DIAGNOSIS

The question of diagnosis concerns two groups of problems: overstatement (over-reporting, positive failures, false positives) and understatement (under-reporting, negative failures, false negatives). Due consideration to both of these important sources of error is rather rarely made in the literature. Steiner (1952) estimates that the error caused by overstatement of cancer is small in U.S. vital statistics, probably less than 5 per cent of reported cancer cases, while understatement may account for as much as 20 per cent especially in internal types of

tumour which are difficult to diagnose. Dorn and Cutler (1955) further discuss these problems. Munck (1952) found that 8 out of 48 autopsied cases of gastric cancer were not diagnosed clinically, whereas the clinical diagnosis could not be confirmed in 5 cases. Hansen (1950), in a thorough study of a Danish hospital material of gastric cancer from the years 1915 to 1935 inclusive, found equally high figures for false positives and false negatives, both amounting to 33 per cent.

With regard to our material, it can be said generally that the city is well equipped with modern hospitals. Since the social insurance covers more than 85 per cent of all people, there are seldom if ever any economical problems concerned with medical care. Easy access to X-ray and laboratory examinations is provided also for patients treated by private practitioners. In later years, autopsy is performed in 50 to 60 per cent of all deaths. Only licenced physicians are allowed to fill in death certificates.

Overstatement.—Although the exact percentage of histologically verified cases cannot be given for our material as a whole at the present time, some valuable information is obtained from the Norwegian Cancer Registry. This instituiton began its activity in 1952, and the project has enjoyed the full support of the medical profession from the very outset. Since no main difference exists between the morbidity and mortality statistics with regard to gastric cancer, an analysis of the registered cases from the two later years of the period may be representative with regard to overstatement. Table II shows that from 93 to 100 per cent of the cases reported from Oslo with the diagnosis of gastric cancer have been hospitalized and that, in 1953, the diagnosis was confirmed by histological examination in 81·1 and 70·7 per cent in males and females respectively. These figures are expected to increase further, since some of the patients are still living. So far, autopsy has been performed in 63 and 53·2 per cent of the deaths. Furthermore, figures were obtained from the mortality statistics of Oslo for one single year within the period (1953). For this year, every death certificate mentioning cancer was matched with the detailed files of the Cancer Registry. This gave the result that the diagnoses of gastric cancer were considered false or inadequate in only six out of 158 cases or in 3·8 per cent (Table III). This may likewise indicate that the degree of overstatement is low in recent years, probably less than 5 per cent.

TABLE II.—*New Cases of Gastric Cancer in Oslo*, 1952–1953

(Source : The Norwegian Cancer Registry)

	1952.		1953.	
	M.	F.	M.	F.
Total number of cases reported	96	72	122	82
Diagnosis confirmed by histological examination‡ — number	—	—	99	58
Diagnosis confirmed by histological examination‡ — per cent	—	—	81·1	70·7
Dead per December 31, 1954	—	—	97	62
Autopsied, number‡	—	—	61	33
Autopsied, per cent	—	—	63·0	53·2
Not hospitalized, all ages	0	4*	2*	7†
Not hospitalized, aged 80 and over	—	3	1	3
Hospitalized, per cent	100	94·4	98·2	93·5

* Positive X-ray in one case.
† Positive X-ray in 3 cases.
‡ This number will increase further since several of these patients are still living.

TABLE III.—*Correction of Cancer Death Certificates, Oslo,* 1953

(Sources: the Norwegian Cancer Registry and the Central Bureau of Statistics, Oslo)

	Total number reported, 1953.	Corrected g.c. to other.	Over-statement per cent.	Corrected other to g.c.	Under-statement per cent.*
Gastric cancer	158	6	3·8	—	—
All cancer	859	—	—	5	3·2

* Per cent of gastric cancer cases.

Understatement, on the other hand, can only be roughly estimated by indirect means. Table III also shows that among 859 of total cancer deaths reported, only five could be referred to overlooked or misdiagnosed primary stomach cancers. A more significant number of false negatives might, however, still be present among the cases recorded as non-cancer on the death certificates.

An attempt was made to secure some information with regard to this point by recording the relative number of unexpected gastric cancer deaths at the Autopsy Department of the University Clinic (Rikshospitalet) in Oslo. Table IV shows that in 21 out of 99 autopsied cases of gastric cancer, or in 21·2 per cent, the diagnosis was missed by the clinicians. The patients treated at this hospital however, are derived mainly from the country districts. Figures were therefore obtained from the two largest municipal hospitals in Oslo (Ullevål and Aker Sykehus). These show (Table V) that the diagnosis was not made clinically in 68 out of 310 autopsied cases, or in 21·9 per cent.

These data are in remarkably good accord with Steiner's figures (1952). As expected, they are lower than those reported by Hansen (1950) whose material partly stems from a period in which the diagnostic facilites were less developed.

TABLE IV.—*Unexpected Gastric Cancer Deaths at Rikshospitalet,* 1948–1954

		Total.	–29.	30–39.	40–49.	50–59.	60–69.	70–79.	80–.	50–79
Number of autopsy cases	M.	70	1	1	9	18	24	17	2	59
	F.	29	1	2	7	4	7	5	1	16
	M.+F.	99	2	3	16	22	31	22	3	75
Diagnosis missed by clinical examination*	M.	13	1	0	4	2	4	2	0	8
	F.	8	1	1	2	0	3	0	1	3
	M.+F.	21	2	1	6	2	7	2	1	11
	M.+F., %	21·2	—	—	—	—	—	—	—	14·7

* If operated, prior to operation.

TABLE V.—*Unexpected Gastric Cancer Deaths at the Municipal Hospitals in Oslo,* 1949–1953.

		Total.	–29.	30–39.	40–49.	50–59.	60–69.	70–79.	80–.	50–79.
Number of autopsy cases	M.	178	0	2	10	39	53	44	30	136
	F.	132	0	3	5	18	32	44	30	94
	M.+F.	310	0	5	15	57	85	88	60	230
Diagnosis missed by clinical examination*	M.	31	0	0	1	7	7	7	9	21
	F.	37	0	0	2	1	6	13	15	20
	M.+F.	68	0	0	3	8	13	20	24	41
	M.+F., %	21·9	—	—	—	—	—	—	—	17.8

* If operated, prior to operation.

As previously mentioned, our material has been restricted to patients aged 50 to 79 years. If a similar restriction is made in the autopsied material from the University Clinic, the negative diagnostic failures are reduced from 21·2 to 14·7 per cent. Similarly, in the material from the Municipal Hospitals, the reduction will be from 21·9 to 17·8 per cent (Table V).

The question arises how far this ratio of understatement in hospital materials may be representative of the degree of understatement which may be present in a material based on death certificates from the city.

Two groups of factors with opposite effects seem to be operative. A hospital material is always selected in the sense that it, of course, includes a relatively high proportion of obscure cases in which a clear-cut clinical diagnosis may be impossible, especially if the patient's general condition is lowered. Since gastric cancer is expected to be a relatively frequent finding in such cases, this will tend to increase the amount of false negatives in any scandinavian hospital material. On the other hand, the better diagnostic facilities in hospitals and the fact that modern surgical departments receive many unquestionable gastric cancer cases for operation will tend to lower the relative amount of negative diagnostic failures. The autopsy frequency at the University Hospital has in later years been about 85 per cent of all deaths occurring at the hospital.

The difference between the hospital materials and that obtained from the death certificates with regard to diagnosis is further reduced by the fact that some two-thirds of the total deaths amongst the population of Oslo actually occur in hospitals and that nearly 60 per cent of all citizens are autopsied at death. We may therefore assume that the ratio of negative diagnostic failures to be expected in the death certificates from Oslo in later years, with regard to gastric cancer, may be in the order of 10 to 20 per cent.

Clearly, some sort of bias might still be introduced in our material in this way. Some tentative information in this respect might be obtained by determining if any social or socio-economic factor is connected with the part of the city population which die in hospitals. By the kind assistance of the Oslo Bureau of Statistics (Östberg, personal commmunication) we have obtained information from two large parts of the city (designated " eastern " and " western " respectively) which, as will be shown later, differ markedly with regard to gastric cancer mortality. Data from the year 1952 show that 68·8 per cent of the total number of deaths from all causes in the population of the eastern zone occurred in hospitals, while the corresponding figure for the western zone was 64·1.

This may be taken to indicate that the risk of understatement connected with lack of hospitalization does not differ significantly in two large regions showing a marked difference in gastric cancer mortality. Thus, we assume that figures which may be obtained from the death certificates from later years in Oslo show a sufficient reliability with regard to overstatement as well as to understatement of gastric cancer.

MOBILITY OF THE POPULATION

Another source of error in investigations on the geographic pathology of cancer may be related to the mobility of the population groups which are to be examined. Since carcinogenic factors in many instances seem to be active in the course of several years, the question may be asked to what extent the pertinent population

groups have in fact been living within the respective locations for a sufficient length of time.

We have chosen 15 (or 13 to 17) years as a reasonable test period in this respect. Consequently, the residences of our patients were recorded for the year 1936 and compared with residences at death. These data were kindly furnished by the Oslo Population Registry.

TABLE VI.—*Residences of Gastric Cancer Patients 15 Years Prior to Death*

Residence at death in Oslo, 1949–1953.		Residence in 1936.			
		Unknown.	Inner Zone.	Outer Zone.	Outside Oslo.
Inner Zone . . 616	.	52	505	20	39
Per cent . . 100	.	8·4	82	3·2	6·3
Outer Zone . . 227	.	24	39	145	19
Per cent . . 100	.	10·6	17·1	63·8	8·4

Table VI shows that only 6·3 per cent of the " Inner Zone patients " and 8·4 per cent of the " Outer Zone patients " in our material were recorded as living outside Oslo in 1936. No adress could be given in 8·4 and 10·6 per cent respectively. Few patients (3·2 per cent) living in the Inner Zone at death had formerly lived in the Outer Zone, whereas movement in the opposite direction had taken place in as many as 17·1 per cent. Thus, it seems as if the material from the Inner Zone is the more stable with regard to this type of intra-city mobility. As mentioned previously, these " Inner Zone patients " will form the main part of our subsequent investigations. On the other hand, further examinations revealed that the mobility between the different districts of the Inner Zone was nearly 30 per cent. It was subsequently shown, however, that movement had largely taken place between districts of a similar socio-economic character.

We therefore conclude that our material is sufficiently well studied as to allow tentative conclusions with regard to socio-economic conditions extending over several years.

SUMMARY AND CONCLUSIONS

A material of gastric cancer has been collected with the intention to avoid or to evaluate some common sources of error with regard to investigations on the epidemiology of the disease.

The population of Oslo offers certain advantages for such studies. The inhabitants are very homogeneous with regard to race, the physio-geographic environment is uniform, the population is well registered, the gastric cancer mortality rate is high (48 and 31 per 100,000 for males and females), and the city shows a rather marked internal social differentiation. Investigation has been made of 843 cases recorded on the death certificates in the five year period 1949–1953.

Figures are presented which tend to show that the degree of overstatement is very low, probably less than 5 per cent, while understatement may amount to 10–20 per cent of the reported cases. This error may be somewhat reduced by confining the material to persons aged 50 to 79. About two-thirds of all deaths in Oslo occur in hospitals. The risk of understatement connected with lack of

hospitalization does not differ significantly in two large regions showing a marked difference in gastric cancer mortality.

The main part of the investigation is concerned with the population of the Inner Zone or " city proper ". Only 6·3 per cent of the patients from this zone were recorded as living outside the city 15 years prior to death, while 3·2 per cent had lived in the Outer Zone. No address could be given in 8·4 per cent. Movement between the different districts of the Inner Zone had taken place in some 30 per cent. Thus, some 50 to 60 per cent of the patients lived at death within the same districts as they did 15 years previously. It was subsequently shown that movement had largely taken place between districts of a similar socio-economic character.

It is therefore believed that the collected material may allow tentative conclusions with regard to certain socio-economic conditions extending over several years.

We are indebted to Magister Egil Nilsen for statistical aid and to the Norwegian Cancer Society (Landsforeningen mot Kreft, Oslo) for economical support.

REFERENCES

DALAFRESNAYE, J.—(1950) Recommendations. In 'Symposium on Geographical Pathology and Dermography of Cancer.' Council for the Co-ordination of International Congresses of Medical Sciences. Oxford (Eng.), 11.

DORN, H. F. AND CUTLER, S. J.—(1955) 'Morbidity from Cancer in the United States. Part I. Variation in Incidence by Age, Sex, Race, Marital Status, and Geographic Region.' U.S. Department of Health, Education, and Welfare, Public Health Monograph No. 29.

DUNHAM, L. J. AND DORN, H. F.—(1955) *Schweiz. Z. Path.*, **18**, 472.

Expert Committee on Health Statistics, Third Report (1952). World Health Organization, Technical Report Series No. 53. Geneva.

HANSEN, J. L.—(1950) Quoted from Clemmesen, J. 'Nye medisinske fremskritt III.' *Berlingska Boktryckeriet*. Lund (Sweden).

MUNCK, W.—(1952) *Acta med. scand.*, Suppl. **261**, 775.

PETERSEN, M.—(1954) 'Slum Clearance and Rehousing in Oslo.' International Federation for Housing and Town Planning. Edinburgh.

SCHMID, C. F.—(1938) 'The Theory and Practice of Planning Census Tracts.' *Sociology and Social Research*, **22**, 228.

STEINER, P. E.—(1952) *Cancer Res.*, **12**, 455.

THE EPIDEMIOLOGY OF GASTRIC CANCER IN OSLO: CARTOGRAPHIC ANALYSIS OF CENSUS TRACTS AND MORTALITY RATES OF SUB-STANDARD HOUSING AREAS

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Received for publication March 15, 1956

CARCINOMA of the stomach remains the principal cause of death from cancer in most countries from which adequate statistics are available. It may be doubted if this sad result of modern therapy can be radically improved even if the diagnosis is made at a reasonably early time (Macdonald and Kotin, 1954). This view, if accepted, means that the size of the research problem (Steiner, 1952) may be particularly large for these types of tumour and that every effort should be made to investigate factors which may be related to their etiology.

Several investigations (Gade, 1916; Registrar General, 1938; Stocks, 1947; Cohart, 1954) seem to be in agreement with the hypothesis that a low socio-economic status may in some way be connected with an increased tendency to develop gastric cancer. Recent investigations from Oslo by Rennæs and Östberg (1955) covering the period 1930–1949 inclusive, also tend to support this view. Patno (1954), on the other hand, studying cancer prevalence in 16 homogenous areas (census tracts) in Pittsburgh, Pennsylvania, arrived at somewhat different conclusions. She found no apparent difference in overall cancer morbidity between typical areas characterized by low and high socio-economic conditions. Furthermore, women of an area showing high social and economic status also experienced high rates for cancer of the digestive organs. Unfortunately, the occurrence of gastric cancer was not specified in this series. Clemmesen and Nielsen (1951) found a rather marked social gradient for gastric cancer in Copenhagen. They felt, however, that its degree of significance did not lend any solid support to its reality, considering the difficulties involved in making the diagnosis.

In a previous paper, we (Torgersen and Petersen, 1956) have emphasized some common sources of error which may be present in statistics on cancer epidemiology. As already stated, an attempt has been made to avoid or evaluate these factors in the present material.

The purpose of the present investigation is (1) to present a cartographic analysis of residences of gastric cancer patients recorded on the death certificates from Oslo 1949–1953 with regard to some indices of socio-economic status, and (2) to present a statistical analysis of gastric cancer mortality in certain sub-standard housing areas ("slum clearance districts") as compared with the rest of the city.

MATERIAL

The material has been presented in our previous article (Torgersen and Petersen, 1956). For the geographic analysis, rates were computed for males aged 50 or

more. Males were selected in order to facilitate comparisons with broad occupational groups. For the statistical analysis, both sexes were considered. For reasons already given, patients aged from 50 to 79 were selected for this investigation.

RESULTS

Cartographic records

Fig. 1 shows the mortality rates for men aged 50 and more according to residence at death. From a cartographic point of view, three main tendencies seem to appear.

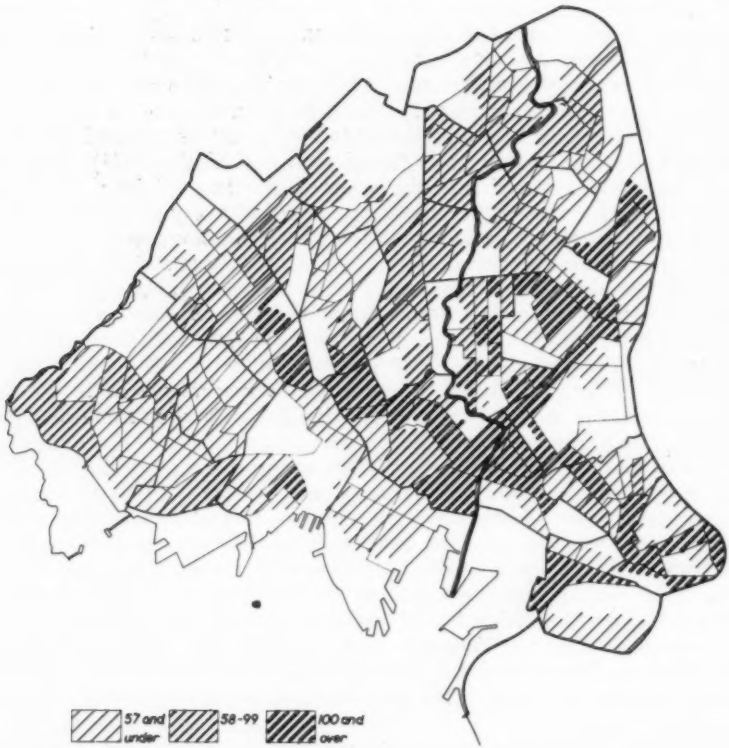

FIG. 1.—Mortality rate from gastric cancer per 10,000 males aged 50 and over, according to residence. Oslo, Inner Zone, 1949–53.

First, the highest death rates seem to prevail in the eastern half of the city. Second, the older residential districts surrounding the main business centre seem to be rather frequently involved. Third, there seems to be a tendency to concentration in areas bordering upon three of the main lines of communication which radiate from the business centre. These lines of communication are characterized as extensions of the business centre.

Fig 2 shows the dwelling density (number of persons per room) in the various areas. Again the east-west division is rather marked, and so are to some extent also the mentioned lines of communication. On the other hand, the areas surrounding the business centre have a medium dwelling density. Rather remarkable is furthermore the fact that a high dwelling density is found in certain northern areas, some of which are characterized by low gastric cancer mortality rates.

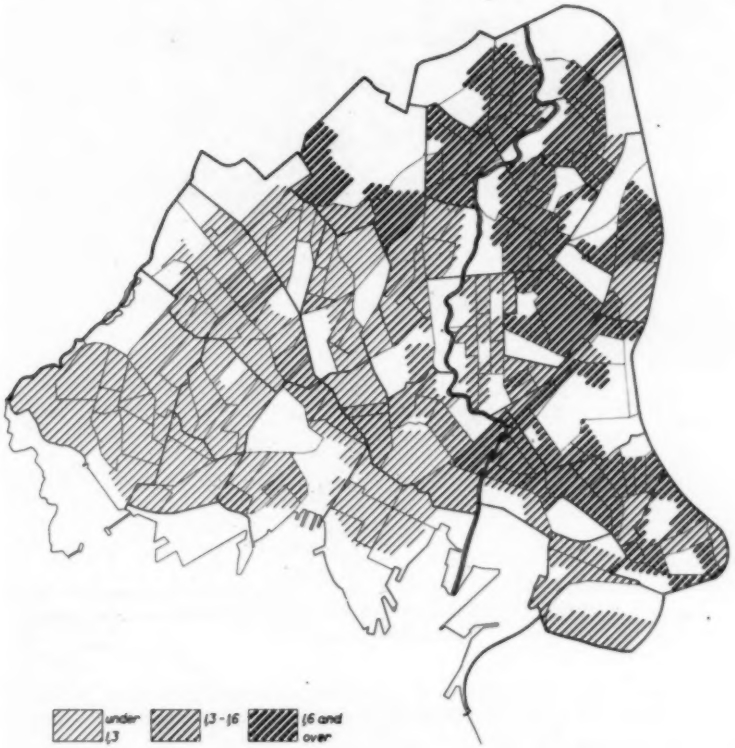

FIG. 2.—Dwelling density (number of persons per room).
Oslo, Inner Zone, 1948.

Fig. 3 shows the distribution of industry. This follows a pattern which roughly recalls the three characteristics of the cancer chart. The main exception seems to be that the centre of the city, which contains rather much " small " industry, has no counterpart with regard to increased gastric cancer mortality. Another seeming discrepancy is the concentration of considerable industry in the northern axial part (along the river " Akerselva " which runs north-south through the city). Part of the corresponding residence districts show, however, a medium mortality rate of gastric cancer.

FIG. 3.—Location of Industry. Factories employing 10 and more workers. Oslo, Inner Zone. 1946.

Fig. 4. shows the distribution of substandard housing areas (" slum clearance districts ") which likewise seem to include many of the districts most heavily afflicted with gastric cancer.

Gastric cancer death rates of sub-standard housing areas

Although a valuable general impression can be obtained from cartographic studies, more precise information must be sought by statistical methods. The beforementioned sub-standard housing areas have been outlined by The Oslo Town Planning Office based on the following criteria : (1) the presence of many appartments condemned or considered condemnable by the Oslo Health Department, (2) high dwelling density, (3) high housing density, (4) the presence of industry and (5) the lack of social institutions in the area (Petersen, 1955).

A further characterization of the sub-standard housing areas compared with the Inner Zone in general is given in Table I. These districts include the residences of 12·2 per cent of the population of the Inner Zone, whereas only 9·9 per cent of

FIG. 4.—Substandard housing areas. Districts given a high rate priority for clearance and renewal of the present housing. Oslo, Inner Zone.

TABLE I.—*Characteristics of Substandard Housing Areas Compared with "Inner Zone" of Oslo*

	Substandard housing areas.	Inner Zone.	Substandard housing areas, % of Inner Zone
Population, 1936	36,350	275,150	13·2
" , 1950	33,129	281,000	12·2
Change in population, 1936/50	−3,221	+5,850	
No. of rooms, 1948	21,854	221,271	9·9
Persons per room	1·52	1·25	
No. of children 0–15 years	5,240	45,950	11·4
Children under public care	138	860	16·1
Youth registered for public care	40	286	14·0
Sentences 1949, drunkenness	28	197	14·2
" , thefts etc.	36	160	22·5
Industry, units 1952*	101	641	15·7

* Employing ten or more workers.

the residence rooms are found in these areas. Furthermore, 16·1 per cent of the children under public care are living in these districts whereas the proportion of all children under 15 years of age is 11·4 per cent. Of particular interest is the fact that as much as 22·5 per cent of all crimes for gain are committed by inhabitants of these " social problem-areas ". There has been a tendency to move out of these districts in later years.

Table II shows the mortality rate of gastric cancer in these areas, by age and sex, as compared with the rest of the Inner Zone and the Outer Zone respectively. The sub-standard housing areas shows an increased gastric cancer mortality in the three recorded age groups. The age- and sex-corrected mortality rate is 14·7 for these districts as compared with 10·0 for the rest of the Inner Zone. This difference is statistically significant ($\chi^2 = 15{\cdot}68$; df = 6; $0{\cdot}01 < P < 0{\cdot}05$).

TABLE II.—*Deaths from Gastric Cancer in Substandard Housing Areas Compared with Rest of Oslo*

		50–59.		60–69.		70–79.	
		M.	F.	M.	F.	M.	F.
Deaths from gastric cancer	Substandard housing areas	7	7	24	9	18	15
	Rest of Inner Zone	52	34	79	55	78	89
	Outer Zone	20	13	35	15	50	32
Population Dec. 1, 1950	Substandard housing areas	2,333	2,690	1,380	1,784	818	1,119
	Rest of Inner Zone	17,345	23,012	9,525	14,534	4,361	8,843
	Outer Zone	7,834	8,091	3,893	4,370	2,652	2,651
Deaths per 10,000 per year	Substandard housing areas	6·0	5·2	34·8	10·1	44·0	26·8
	Rest of Inner Zone	6·0	3·0	16·6	7·6	35·8	20·2
	Outer Zone	5·1	3·2	18·0	6·9	37·7	24·2

DISCUSSION

The data presented in the cartographic analysis are generally in good conformity with the " concentric zone hypothesis " of Burgess (1925) according to which the most characteristic " slums " are to be found in areas surrounding business districts (Petersen, 1956). The inhabitants of these areas seem to be particularly exposed to the risk of developing gastric cancer.

On the other hand, certain objections may be mentioned.

First, the difficulties connected with the collection of relevant data are evident. Thus, it has been argued that some patients might have got into economical troubles and might possibly have moved into " slum " areas because of bad health such as gastro-intestinal disturbances which, in some cases, might have been gastric cancer or some possible precursor such as gastritis, gastric ulcer, polyps or pernicious anemia. This would imply that the low socio-economic status would be the effect rather than a (contributory) cause of the disease in some cases. The fact that most patients in our material have been living within the same areas for more than 15 years, however, may be a certain extent invalidate this argument. This and some other sources of error have been dealt with in our previous article (Torgersen and Petersen, 1956).

Second, the cartographic analysis apparently shows the limited value of using such crude correlates as " dwelling density ", " industry " etc., in such investiga-

tions. Thus, for instance, a most outstanding discrepancy is observed in certain districts with regard to dwelling density. Although a positive correlation between this factor and gastric cancer death rate seems to exist, some of the areas presenting a high dwelling density show a low rate of gastric cancer (some northern districts in Fig. 2, as compared with Fig. 1). Part of the explanation, however, may be that the high dwelling density in these districts is mainly due to the presence of many children which probably is a factor of negligible significance with regard to gastric cancer mortality. This point will be studied more closely in subsequent work.

The effect of socio-economic factors connected with residence in sub-standard housing areas (" slum clearance districts ") is indicated by the increased mortality rate of gastric cancer in these districts as compared with the rest of the Inner Zone.

It must be admitted, however, that the factor groups at present available for correlation with gastric cancer mortality are very crude. Thus, the transcription of " low socio-economic conditions ", " high dwelling density ", etc., into terms of etiology such as deficient nutrition or the ingestion of carcinogenic substances would seem a rather hazardous task at the present time. In further investigations, other factor groups of possible significance will be registered, and attempts will be made to transform some of these problems into the experimental field.

SUMMARY

An analysis has been made of the deaths from gastric cancer recorded on the death certificates of Oslo during the five-year period 1949–1953. Particular attention has been paid to various sources of error, as described in a previous article (Torgersen and Petersen, 1956).

Two types of investigation have been performed, namely cartographic analysis of small areas (" census tracts "), and comparisons between gastric cancer death rates of sub-standard housing areas (" slum clearance districts ") and the rest of the city.

The cartographic analysis outlines certain areas which show a particularly high death rate from gastric cancer (over 100 per 10,000 ; Fig. 1). Comparisons are made with location if industry, dwelling density, and sub-standard housing areas (Fig. 2–4).

The latter districts reveal a mortality rate from gastric cancer amounting to 14·7 as compared with 10·0 for the rest of the Inner Zone. This difference is significant statistically. A further characterization of these districts is given in Table I.

These investigations are consistent with the hypothesis that socio-economic factors are effective in the genesis of gastric cancer. The need for a more precise definition of such factors is emphasized.

We are indebted to Magister Egil Nilsen for statistical aid and to The Norwegian Cancer Society (Landsforeningen mot Kreft, Oslo) for economical support.

REFERENCES

Burgess, E. W.—(1925) ' The Growth of the City : An Introduction to a Research Project.' Chicago.

Clemmesen, J. and Nielsen, A.—(1951) *Brit. J. Cancer*, **5**, 159.

COHART, E. M.—(1954) *Cancer*, **7**, 455.
GADE, F. G.—(1916) ' Undersökelser over Kraejtsygdommene i Norge.' *Videnskaps-selskapets Skrifter I. Mat. naturv. klasse*, **7**, 76.
MACDONALD, I. AND KOTIN, P.—(1954) *Surg. Gynec. Obstet.* **98**, 148.
PATNO, M. E.—(1954) *Publ. Hlth Rep. Wash.*, **69**, 705.
PETERSEN, M.—(1955) ' Oslos geografiske struktur.' (Mimeographed, Oslo Town Planning Office.)
PETERSEN, M.—(1956) ' The Urban Fringe and Metropolitan Structure.' International Federation for Housing and Town Planning, Wien.
REGISTRAR-GENERAL—(1938) Decennial Supplement, England and Wales, Part IIa. ' Occupational Mortality, 1931.' London (H.M. Stationery Office).
RENNAES, S. AND ÖSTBERG, E. W.—(1955) *Brit. J. Cancer*, **9**, 7, 20.
STEINER, P. E.—(1952) *Cancer Res.*, **12**, 455.
STOCKS, P.—(1947) ' Regional and Local Differences in Cancer Death Rates.' London (H.M. Stationery Office).
TORGERSEN, O. AND PETERSEN, M.—(1956) *Brit. J. Cancer*, **10**, 292.

REACTIONS IN LYMPH NODES DRAINING X-IRRADIATED AND CARCINOGEN TREATED TISSUES

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Received for publication March 23, 1956

A NUMBER of authors have described changes in lymphoid tissue after irradiation or exposure to carcinogenic agents. These reactions appear to be of two distinct types. Firstly, formation of so-called "haemo-lymph nodes" may occur. This change has been described, among others, by Clarkson, Mayneord and Parsons (1938) following whole body X-irradiation in mice, and by Lasnitski and Woodhouse (1944) following treatment with a number of carcinogens. It is probable that the increase of iron pigment observed by Hoch-Ligeti (1941) in the nodes of mice treated with 1, 2, 5, 6-dibenzanthracene was a late stage of this effect.

The second change consists of the formation of plasma cells throughout the lymphoid tissue. This reaction has been reported following whole-body exposure to ionizing radiation in man (Liebow, Warren and De Coursey 1949), in dogs (Wohlwill and Jetter, 1953), in monkeys (Schlumberger and Vazquez, 1954), in mice following irradiation and also after treatment with a water soluble carcinogen (Parsons, 1943), and in the mesenteric nodes of mice following intraperitoneal injection of carcinogenic hydrocarbons (Green, 1954). In view of the known connection between plasma cell proliferation and antibody formation (Fagraeus, 1948), the last change was interpreted by Green to represent an immune reaction against altered protein formed by the carcinogen and to be intimately connected with the subsequent process of tumour formation.

In this paper the reaction of the regional lymph nodes to local treatment with carcinogens and X-rays was studied in mice and guinea-pigs at varying periods from the time of application of the agents.

METHODS

Carcinogens

9, 10-Dimethyl-1, 2-benzanthracene and 20-methylcholanthrene dissolved in acetone were employed.

Fifty Swiss albino mice were given weekly paintings with a 0·15 per cent solution of 9, 10-D.M.B.A. with 0·2 per cent methylcholanthrene on the right foreleg, thoracic and axillary regions. These animals were killed at intervals of from 14 days to 14 weeks from the first painting. The right and left axillary groups of lymph nodes were removed, weighed, and retained for histological study. Superficial ulceration of the skin of the treated area occurred in 8 of 50 animals, and tumour formation was present in 7 of the 10 animals surviving at 14 weeks.

A group of 8 animals was also given a single painting with 0·4 per cent 9, 10-D.M.B.A. and sacrificed 14 days later. Severe skin damage occurred in these animals.

X-rays

Group 1.—Five female guinea-pigs were given a total of 9,000r of X-rays (50 KV., 3 Ma., H.V.L. 0·33 mm. Al., 4 cm. field, 4 cm. distance, 3,500r per min.) in 5 separate doses of 1,500r at 2–3 week intervals, on the dorsum of one foot and were killed 10 days after the last exposure.

Group 2.—Five female guinea-pigs were also given a single exposure on one foot of 2,900r and a second exposure of 2,800r after a 5-week interval. They were killed from 4 to 10 days after the second exposure. Following the second exposure ulceration occurred in 4 of 5 animals ; in this group such animals were killed within 48 hours of the appearance of ulcers.

In mice treated with chemical carcinogens the axillary lymph nodes on both the treated and control sides were removed, weighed and fixed in formol alcohol. In irradiated guinea-pigs, the popliteal and femoral lymph glands on the irradiated and normal sides were similarly removed. Portions of the carcinogen-treated and irradiated skin were also preserved for histological study. Paraffin sections from this material were stained by haematoxylin and eosin, Unna-Pappenheim's method and Perl's method for iron.

RESULTS

All mice treated with 20-methylcholanthrene and 9–10 dimethyl benzanthracene, and killed from 14 days to 14 weeks, showed a marked increase in weight of the axillary lymph nodes on the treated side (Table I). This increase was greatest in the 7 animals bearing tumours after 14 weeks' treatment.

TABLE I—*Total Weights of Axillary Lymph-nodes from Control and Treated Sides in Mice Painted for Varying Periods with Carcinogen.*

Carcinogen.	Duration (days).	Treated weight (mg.).	Control weight (mg.).
D.M.B.A.	14	430	270
D.M.B.A.	21	320	160
D.M.B.A.	35	205	100
20-methylcholanthrene	42	170	70
20-methylcholanthrene	98	160	60
20-methylcholanthrene (tumour bearing)	98	450	180

Histological Changes in Lymph Nodes Draining Carcinogen Treated Areas

These reactions were identical whether 20-methylcholanthrene or 9, 10-dimethyl-benzanthracene were used. They may be described under two headings.

(1) *Reactions in cortical and medullary pulp of nodes*

These reactions developed within 14 days of the start of treatment and showed only a moderately greater degree in animals killed after long periods of treatment. The main proliferative reaction was in the medullary pulp of the node and showed

in its earlier stages a diffuse proliferation of cells with pale oval nuclei and inconspicuous non-basophilic cytoplasm (Fig. 1). These cells resemble the " primitive reticular cells " described by many authors as being the common stem cell observed in lymphoid and myeloid tissues. In material studied after longer periods of treatment, fewer cells of the primitive reticular type were present and a considerable degree of differentiation to small and medium lymphocytes appeared to have occurred (Fig. 2). The resulting proliferation produced a considerable widening of the medulla of the node with some compression and narrowing of the medullary cords near the hilum. In non-tumour bearing animals treated with carcinogens from 14 days to 7 weeks there was no evidence of plasma cell proliferation in the medullary cords or medullary pulp of the node, and the few plasma cells of mature type present in the medullary cords did not exceed that observed in the control node from the opposite side. In 3 mice (non-tumour bearing) treated for 14 weeks with methylcholanthrene, however, a moderate plasma cell increase was present in the medullary cords of the treated node.

The formation of germinal centres, which forms a prominent part of the lymph node reaction to antigens, was not conspicuous in the treated animals ; hyperplastic centres were present in both control and treated nodes of some animals, and were absent in treated nodes in others.

In 7 tumour-bearing animals treated with methylcholanthrene a moderate to heavy plasma cell proliferation was present in the medullary cords of the treated nodes (Fig. 3) in addition to the lymphocytic medullary proliferation previously described.

(2) *Reactions in sinuses of treated nodes*

These were similar to those previously described by Lasnitski and Woodhouse (1944). A moderate proliferation of sinus histiocytes occurred, and in some animals red cells were present, either free or intracellularly. Free iron could also be demonstrated in the cytoplasm of the histiocytes in many treated animals. This change was not confined to the nodes draining the treated area but was also present in control glands from the opposite axilla.

Histological Changes in Lymph Nodes Draining X-irradiated Tissue

Group 1

In 5 guinea-pigs given a total of 9,000r of X-rays in 5 doses of 1,500r, the popliteal and femoral nodes draining the radiated foot showed only slight histological changes. Slight proliferation of sinus lining histiocytes was present in all examples, in one animal considerable phagocytosis of red cells was present. A moderate hyperplasia of the lymphoid tissue of the medulla occurred in all animals. This apparently produced only cells of the lymphocyte series, and resembled the proliferation seen in carcinogen treated animals. A small proliferation of mature plasma cells was present in the medullary cords in the popliteal node of one animal.

Group 2

In the 5 guinea-pigs given a single exposure of 2,900r, followed after 5 weeks by a second exposure of 2,800r, more marked histological changes were present in the draining lymph nodes. A similar sinus proliferation to that seen in Group 1

was present, and polymorphonuclear leucocytes, red corpuscles and a few eosinophils were present. Lymphoid proliferation similar to Group 1 was also present in the medulla of the node (Fig. 4). A marked plasma cell proliferation occurred in the medulllary cords of the lymph nodes of 4 animals (Fig. 5). Plasma cells of immature type were present in these proliferations, some showing mitotic figures.

DISCUSSION

Of the various reactions described in the treated lymph nodes, that of deposition of iron pigment or phagocytosis of red cells appears to correspond with that previously described by a number of authors. This reaction would appear to be a consequence of local or general capillary damage by X-irradiation or chemical carcinogens with subsequent leakage of red cells into the tissue spaces.

The hyperplasia of stem cells with subsequent lymphocytic differentiation which occurred in the medulla of nodes draining both X-irradiated and carcinogen-treated tissues does not appear to have been previously observed. The reaction resembles that seen in human nodes following a variety of infective and inflammatory stimuli, but in view of present ignorance of the functions of lymphocytes in such processes, no assessment of its significance is possible.

The generalized formation of plasma cells in lymphoid or myeloid tissue has been described following exposure to irradiation or general absorption of an injected carcinogen. It is known that considerable bacteraemia may follow whole-body irradiation, and in view of the frequently observed production of plasma cells by antigen injection it is possible that the plasma cell proliferation in irradiated animals is a secondary consequence of bacteraemia. A similar mechanism may account for the production of plasma cell proliferation in animals following parenteral injection of carcinogens. In these experiments, in which both small and heavy doses of carcinogen were applied to the skin, there was no satisfactory evidence of plasma cell proliferation in the draining lymph node; such proliferation was present however in animals bearing tumours in the treated areas.

The formation of plasma cells as a local response to ionizing radiation has been reported as a sequela of radiotherapy of tumours in man, where it forms part of the so-called "stromal reaction". Some authors (Koller, 1947) have considered this reaction to be of considerable importance in determining the response of the tumours to radiotherapy. The significance of this reaction is uncertain as some degree of infection of the tumour might produce a similar result. In the experiments reported here, plasma cell proliferation was observed in some instances in draining lymph nodes following local exposure to too heavy doses of X-rays and in which

EXPLANATION OF PLATE.

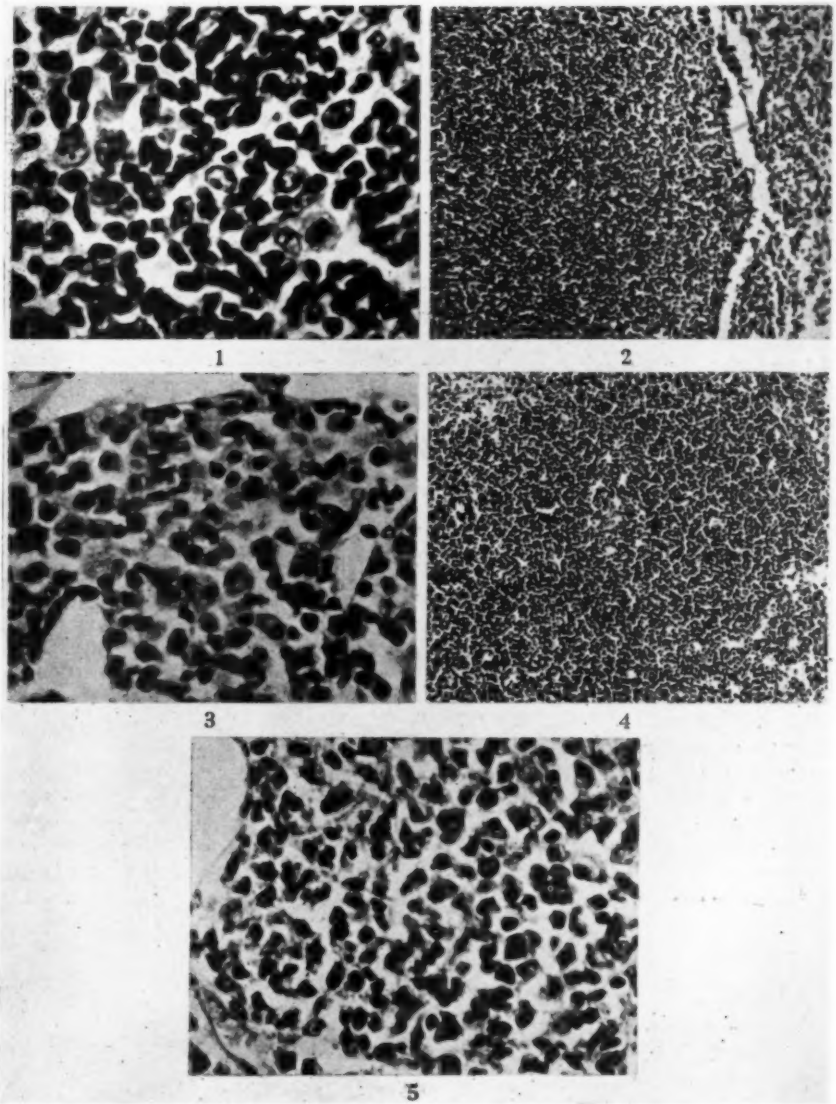
FIG. 1.—Reaction in medulla of regional lymph node after 21 days treatment with methylcholanthrene. Diffuse proliferation of primitive reticular cells, and more mature lymphocytic elements. H. & E. × 700.

FIG. 2.—Late stage of reaction in medulla of methylcholanthrene treated node. The proliferation of mature lymphocytes is compressing the remaining sinus tissue of the node. H. & E. × 120.

FIG. 3.—Plasma cell reaction in medullary cords of regional node in a tumour-bearing animal. No metastasis was present in the node. H. & E. × 500.

FIG. 4.—Proliferation of small and medium lymphocytes in medulla of lymph node draining X-irradiated area of guinea-pig foot. H. & E. × 120.

FIG. 5.—Plasma cell proliferation in medulla of guinea-pig lymph node draining X-irradiated foot. H. & E. × 450.



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local irradiation damage was most severe, but was not seen when a larger total dose was given in 5 fractionated amounts. These results do not show any constant plasma cell response to local irradiation of normal tissue but suggest that such a response may occur when severe necrosis of tissue takes place either as a consequence of antigenic substances derived from damaged tissue or from bacterial contamination of the radiated area.

SUMMARY

In 58 albino mice given weekly paintings on one side of the chest and axilla with 9, 10-dimethyl-1, 2-benzanthracene or 20-methylcholanthrene, an enlargement of the regional nodes, characterized by proliferation of lymphocytic cells in the medulla, was constantly produced. There was no evidence of plasma cell proliferation in response to carcinogen treatment alone, although some such proliferation occurred in animals bearing tumours.

In 10 guinea-pigs treated with multiple doses of X-rays to one foot, a similar lymphocytic hyperplasia was observed; plasma cell proliferation also occurred in the regional nodes in animals in which severe local tissue necrosis had occurred.

REFERENCES

CLARKSON, J. R., MAYNEORD, W. V. AND PARSONS, L. D.—(1938) *J. Path. Bact.*, **46**, 221.
FAGRAEUS, A.—(1948) *Acta med. scand.*, Suppl. 204.
GREEN, H. K.—(1954) *Brit. med. J.*, **2**, 1374.
HOCH-LIGETI, C.—(1941) *Cancer Res.*, **1**, 484.
KOLLER, P. C.—(1947) *Brit. J. Radiol.* Suppl. 1, p. 84.
LASNITSKI, A. AND WOODHOUSE, D. L.—(1944) *J. Anat. Lond.*, **78**, 121.
LIEBOW, A. A., WARREN, S. AND DE COURSEY, E.—(1949) *Amer. J. Path.*, **25**, 853.
PARSONS, L. D.—(1943) *J. Path. Bact.*, **55**, 397.
SCHLUMBERGER, H. G. AND VAZQUEZ, J. J.—(1954) *Amer. J. Path.*, **30**, 1013.
WOHLWILL, F. J. AND JETTER, W. W.—(1953) *Ibid.*, **29**, 721.

RADIOBIOLOGY OF THE C3H MOUSE MAMMARY CARCINOMA: INCREASED RADIOSENSITIVITY OF THE TUMOUR INDUCED BY INOCULATION OF THE HOST WITH RADIATION-ATTENUATED ISOGRAFTS

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Received for publication February 11, 1956.

IN a previous communication (Cohen and Cohen, 1954*b*) it was noted that tumours arising from viable grafts of the C3H mammary carcinoma, which had been attenuated by sublethal doses of radiation immediately before implantation, were found, when subsequently irradiated *in situ*, to have a significantly increased radiosensitivity. The radiosensitization did not appear to be due to any permanent change in the tumour, or to any local reaction in the tumour bed, but could be explained only on the basis of a subliminal systemic resistance mechanism, possibly in the nature of a circulating iso-antibody arising in the host. If this postulate is correct, it might be possible to enhance the radio-sensitivity of an established non-attenuated isograft by inoculation of the host with a suitably attenuated second implant. The present investigation demonstrates that induced radiosensitization of an isogenic tumour in inbred mice is feasible, and may possibly point the way to more efficient therapy of autogenous growths.

The procedure is complicated by additional variables not encountered when the radiation-attenuated homografts themselves are treated. The attenuation doses can vary widely, and may include a higher dosage range where the irradiated inocula no longer "take". One may also choose almost any interval between implantation of the unmodified tumour and the inoculation of the irradiated fragment. Further, the time between the dates of implantation of the attenuated inoculum and of the treatment *in situ* of the established tumour may vary widely. The present experiment, therefore, is designed firstly to explore the possibility of radiosensitization of an unmodified transplanted C3H mammary adenocarcinoma by means of an attenuated tumour inoculum, and secondly to seek effective combinations of timing and dosage factors for this type of procedure.

MATERIALS AND METHODS

Experimental design

Young adult male mice from the C3H/Cg substrain were used. Each received two inoculations: one consisting of fragments of an unmodified inoculum, that is the usual isogenic C3H mammary tumour homoplast, into the right flank, and the second an attenuated inoculum, tumour fragments irradiated *in vitro* prior to implantation, into the left flank. After a suitable period, depending on the experimental variables, the unmodified tumour was irradiated *in situ* using the standard procedure. It was considered that the following variables might affect

results sufficiently to warrant analysis : the attenuation dose, the interval between inoculation of the attenuated implant and of the unmodified tumour, and the interval between inoculation of the attenuated implant and irradiation of the unmodified tumour.

Since the immediate object of this investigation was merely to select, from many possible permutations, a fairly effective radiosensitizing procedure, a complete probit assay was not attempted, but as each unmodified inoculum reached a size of about 1 cm. in diameter, it was irradiated *in situ* with a standard dose of 4200r. This quantity was chosen since the expectation of cure at this dose is normally under 0·1 per cent, but it nevertheless affords very significant cure-rates in those instances when some degree of host resistance operates against the tumour (Cohen and Cohen, 1954*a*). Concurrently, in a control series of 97 mice, each bearing a single unmodified isograft treated at the same dose, no cures were in fact observed.

Irradiation technique

As in all previous experiments of this series, röntgen rays generated at 240 kVp., no added filters, HVL 0·34 mm. Cu., FSD 25 cm., were used. For irradiation *in situ*, a 2 cm. diameter field, directed through the tumour retracted on to a wax block, gave a dose rate of 500r/min. The attenuation procedure was carried out by irradiation of minced tumour fragments in a plastic welled slide with the same technical factors but using a 5 cm. diameter field giving 600r/min. with full back-scatter, as described in our previous communication (Cohen and Cohen, 1953).

Attenuation of the tumour

Using the technique described above, a fairly wide range of dosage from 2500r to 5000r was tested, but for the purpose of analysis, the attenuated inocula were grouped into the two categories shown in Table I, according to their ability to " take " subsequently. In the dose range 2500r to 3000r, the majority of the attenuated implants were viable and grew readily after a moderately prolonged latent period, while in the 3500r to 5000r range, the growth of almost all implants was arrested. Although most implants in the upper range of attenuation dosage do not " take ", there is nevertheless no reason to believe that they are inviable (Cramer, 1932 ; Goldfeder, 1940), so the terms " viable " or " arrested " will here be used to describe attenuated inocula which " take " or fail to " take " respectively. In the case of viable inocula, there is the additional complication that the attenuated tumour has to be removed either by excision or irradiation if the animal is to survive long enough for the unattenuated tumour to be observed and followed.

It has become necessary, in the light of the larger number of attenuations in the present series to revise our 1953 estimate of the LD_{50} for irradiation *in vitro*. The variation among different batches of attenuated tumour has now been found to be much larger than that among individual mice inoculated with the same batch of tumour tissue. Individual " takes " within each group were therefore not stochastically independent of one another, so that each batch of irradiated tumour afforded only one degree of freedom instead of 10 to 15 as previously assumed, giving a standard error much larger than that reported. While not affecting any conclusions drawn (Cohen and Cohen, 1954*c*), the corrected LD_{50} for irradiation prior to implantation now appears to be 3200 ($\pm$ 125)r, instead of 2850r as originally reported (Cohen and Cohen, 1953). In general, the tumour

remained viable when attenuated with doses smaller than this median value, and was arrested by larger doses.

Timing procedures

While the interval between the attenuated and unmodified inocula could be deliberately selected, the time elapsing between inoculation and treatment was not easily controlled and varied widely. Four different attenuation-timing factors were tested : the attenuated inoculum (A.I.) being given 3 weeks before, 3 days before, 3 days after, or 3 weeks after the unmodified inoculum (U.I.), that is, U.I.–A.I. intervals of − 21, − 3, + 3, and + 21 days respectively as shown in Table I. The time intervals between the implantation of the attenuated inoculum and irradiation of the unmodified tumour *in situ* could not be limited to set standards, and ranged from 3 to 60 days, according to the time of inoculation and the tumour growth rates. The last four columns of Table I show the range of this variable and the median values for cured and non-cured mice in each category.

RESULTS

In Table I the results of several individual experiments, in which all tumours were treated with 4200r, are summarized. In the three groups where the attenuated inoculum was given *before* the unmodified tumour graft, there was no response to treatment at this dose level. On the other hand, when the attenuated inoculum *followed* the unmodified implant, the same dose resulted in 26 cures among 92 mice treated, assessed 3 months after irradiation. There seems to be no significant difference between the 3-day and the 21-day U.I.–A.I. interval, given the correct order of inoculation. It is also noted that mice receiving isografts attenuated at 3500 to 5000r, which did not subsequently " take ", showed a much higher cure rate (19/38) than those which had received inocula of viable attenuated tumour (7/54). In 28 of the 54 mice bearing bilateral tumours, the attenuated " take " appeared only after it had become obvious that the unattenuated tumour was not responding to treatment, so that no further intervention was necessary. In 15 cases, of which 4 were cured, the attenuated inoculum was surgically excised between 3 and 26 days after treatment of the unmodified tumour. In 11 cases the attenuated " take " was irradiated with 4200r *in situ* either at the time of treatment of the unmodified tumour, or from 3 to 29 days thereafter ; 3 of these mice were bilaterally cured. There seemed to be no obvious difference in timing between the 7 cured and the 19 recurrent cases in these groups. The greater proportion of cures occured when the interval between the attenuated inoculum and the treatment *in situ* of the unmodified tumour was in the region of 4 to 5 weeks, the effective period apparently being not less than 2 weeks and continuing up to about 7 weeks.

Comparing the 3-month result for this whole experimental series (26/123) cures with the observed control series treated at the same dose (0/97 cures) indicates a highly significant increased radiocurability at the probability level $p < 0{\cdot}00001$.

Over half of the 3-month cures developed late recurrences, a phenomenon not previously observed in this series of experiments. In order to complete the data, therefore, it was necessary to continue follow-up on treated animals, whenever possible, for at least 6 months. Accordingly, both 3-month and 6-month results are shown in Table I ; the former give a useful comparison with previous experi-

TABLE I.—*Effect of Inoculation of the Host with Radiation-attenuated Homologous Tumour Tissue on the Radiosensitivity of the C3H Mouse Mammary Carcinoma Treated* in situ *with* 4200*r*.

U.I.–A.I.* interval. (Days.)	Cure rates of unmodified tumours when attenuated implants are: (*a*) Viable Inocula (2500–3500r). No. of mice.	(*a*) No. cured.	(*b*) Arrested Inocula (3500–5000r). No. of mice.	(*b*) No. cured.	Cure rates after 4200r. (At 3 months) Total.	Per cent.	(At 6 months) Total.	Per cent.	Attenuated inoculum to treatment intervals (days). Cures. Range.	Median.	Non-cures. Range.	Median.
−21	10	0	—	—	0/10	0	—	—	—	—	44–50	47
− 3	10	0	11	0	0/21	0	—	—	—	—	21–60	43
+ 3	34	7	28	15	22/62	36	8/62	13	14–52	35	14–45	40
+21	20	0	10	4	4/30	14	2/30	7	17–34	28	3–34	7
Total	74	7	49	19	26/123	21	} Significant at $p < \cdot 00001$					
Controls	No attenuation				0/97	0						

* U.I. = Unmodified, non-attenuated tumour inoculum.
A.I. = Radiation-attenuated tumour inoculum; see text.

ments of this series in which results were finally assessed at 3 months, and the latter serve to indicate the absolute curability of the tumour under the conditions of the present experiment. There were no recurrences later than 6 months after treatment. There were no obvious differences in timing or technique to account for the long-term cures except that they were confined to those mice in which the attenuated inocula had remained arrested, all of the 7 mice "cured" at 3 months but having viable attenuated inocula developing late recurrences.

From the information available in Table I, it would seem that optimal effects are obtained when attenuated isografts are irradiated *in vitro* with arresting doses in the 3500 to 5000r range, and inoculated between 3 and 21 days after implantation of the unmodified tumour graft, and a period of 2 to 7 weeks allowed to elapse before treatment *in situ*. The cure rate, assessed at 3 months, then approaches 50 per cent compared with an expectation of only 0·1 per cent in the corresponding controls.

DISCUSSION

It has been shown that a significant increase in radiosensitivity appears, firstly, when a tumour which has arisen from an implant attenuated with radiation immediately prior to inoculation is irradiated *in situ*, and secondly, in the treatment of unmodified tumours growing in hosts who have been inoculated concomitantly with an attenuated fragment of the same tumour. While the former category is of limited academic interest, the second effect is of more practical importance in that induced radiosensitization of established tumours may have possible clinical applications.

The cure rate attained in the case of such indirectly radiosensitized tumours is of the same order as that previously reported for directly attenuated tumour inocula treated at the same dose, at best about 50 per cent cures at 4200r. It

seems probable that a similar mechanism operates in the two cases. Previous observations (Cohen and Cohen, 1954*b*, 1954*c*) suggested that the attenuated tissue had been rendered antigenic to the host, thus evoking some generalized systemic resistance mechanism, most probably a circulating iso-antibody. These subliminal immune reactions are apparently regularly associated with an increased radiosensitivity of the tumour (Cohen and Cohen, 1954*a*). The results of the present experiment follow logically from, and lend strong support to this immunological hypothesis.

The tendency to late recurrence in many of the initially cured mice has not been previously observed in this laboratory, although a very large series of cured mice has now accumulated from many preceding experiments. It seems that the apparently cured tumours are not entirely eradicated by irradiation *in situ*, at least at this relatively ineffective dose level, but are subject to some continuing restraint or resistance on the part of the host. The phenomenon of prolonged tumour dormancy associated with host resistance, and recrudescence when this resistance fails, is also well-known in clinical cancer (Hadfield, 1954). The restraining process, perhaps the titre of the antibody, in common with most other immunological responses, tends to diminish with time, thus permitting late, slow-growing recurrences of tumours that were impalpable for some 3 months after treatment.

If the foregoing results are to be explained on an immunological hypothesis, then more effective immunizing procedures are likely to give still better responses. In line with past experience in immunology it may well be that animals bearing primarily unmodified tumours, to which they normally have little or no resistance, may require repeated " booster " inocula of attenuated tissue to maintain the remission. It is possible, too, that tumour tissue attenuated at doses higher than those used in the above experiments would be more effective, as the observed trend towards greater curability with larger attenuating doses was not fully explored.

SUMMARY

C3H mice bearing isografts of the C3H mammary adenocarcinoma were inoculated with fragments of the same tumour that had been attenuated with varying doses of röntgen rays immediately prior to implantation. The originally established tumours were then irradiated *in situ* with 4200r, a dose at which the expectation of cure is normally under 0·1 per cent. In this experimental series, however, the cure rate at 4200r approached 50 per cent, indicating that the procedure had significantly increased the radio-sensitivity of the unmodified isogenic tumour. Optimal results were obtained when the attenuation dose was 5000r, the interval between implantation of the unmodified tumour and the attenuated inoculum was 3 to 21 days, and the interval between the attenuated inoculum and treatment *in situ* was 2 to 7 weeks.

A tendency to late recurrence was noted in some of these tumours apparently cured for 3 months after treatment.

It is considered that inoculation of the attenuated tumour fragment induces a subliminal and transient resistant state in the host, which enhances the radiosensitivity of a previously established tumour.

The authors are deeply indebted to Dr. J. F. Murray, of the South African Institute for Medical Research, who has generously provided all the necessary

facillities for the maintenance of the animals used in these experiments, and to Mrs. Shelley Jacobson for her expert assistance in this work.

REFERENCES

COHEN, A. AND COHEN, L.—(1953) *Brit. J. Cancer*, **7**, 231.—(1954*a*) *Ibid.*, **8**, 303.—(1954*b*) *Ibid.*, **8**, 313.—(1954*c*) *Ibid.*, **8**, 522.
CRAMER, W.—(1932) *Sci. Rep. Cancer Res. Fd., Lond.*, **10**, 95.
GOLDFEDER, A.—(1940) *Radiology*, **35**, 210.
HADFIELD, G.—(1954) *Brit. med. J.*, ii 607.

SERUM ISOMERASE IN LEUKEMIA

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Received for publication April 30, 1956

Bodansky (1954*a*) recently described a method for the determination of serum isomerase and examined the extent to which the serum enzyme activity can be correlated with clinical and biochemical evidence of tumour growth. He found good correlation in breast and prostatic cancer (Bodansky, 1954*b*, 1955).

In view of the considerable isomerase activity shown by erythrocytes and leukocytes, we have determined the serum isomerase concentration in diseases involving the cellular elements of the blood.

MATERIALS AND METHODS

The serum phosphohexose isomerase activity was determined according to the method of Bodansky (1954*a*). This determination was carried out on fresh serum obtained from normal controls, patients with various non-malignant diseases with normal leukocyte counts, patients with chronic myelocytic and chronic lymphocytic leukaemia, and patients with leukocytosis due to infection. Many of the leukemia group were under treatment at the time of isomerase estimation. Those patients with chronic myelocytic leukemia were receiving radiation or dimethylsulphonoxylbutane (Myleran®), and those with chronic lymphocytic leukemia, radiation or *p*-(Di-2-chloroethylamino)-phenylbutyric acid (CB1348).

RESULTS

Fig. 1 shows graphically the values obtained. The results shown in the myelocytic and lymphocytic groups represent the initial value obtained in the particular patient, as in the chronic myelocytic leukemia group subsequent values fell as therapy was instituted or continued.

The values obtained by us in the normal and non-malignant disease groups are in reasonably good agreement with those of Bodansky who found a mean of 20 and a standard deviation of 8 in the former and a mean of 18 and a standard deviation of 5·5 in the latter. Our results, however, show a greater spread downwards towards lower values.

Fig. 1 also demonstrates the high values in chronic myelocytic leukemia as compared with the other groups. The lowest values in this group were obtained from patients who had been on Myleran therapy for many months prior to the isomerase estimation.

Fig. 2 shows the relationship between the serum isomerase and the number of cells of the myelocytic series in chronic myelocytic leukemia before and after treatment, chronic lymphocytic leukemia and infection. In the chronic myelocytic

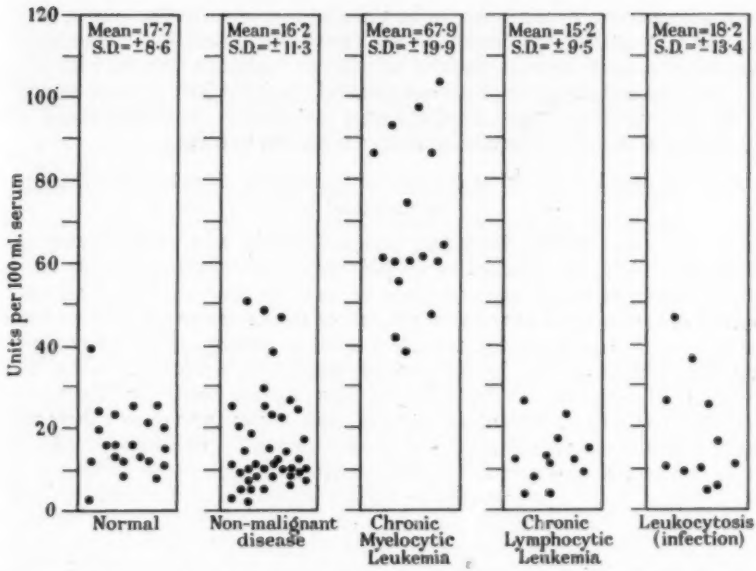

FIG. 1.—Initial serum isomerase levels in normal controls, non-malignant disease, chronic myelocytic and chronic lymphocytic leukemia and leukocytosis due to infection.

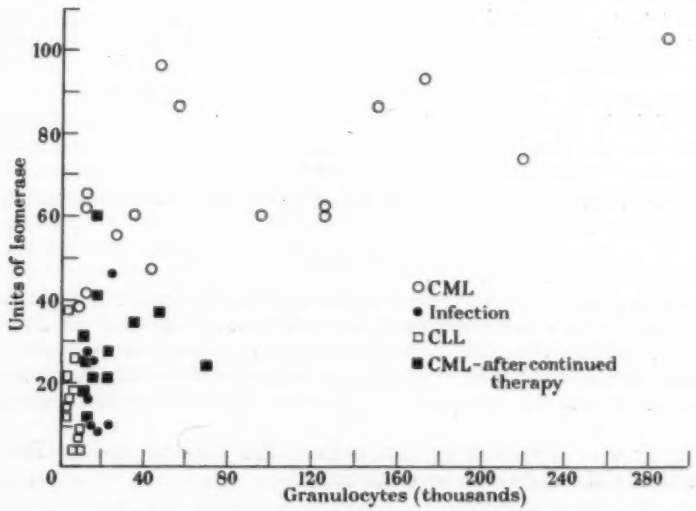

FIG. 2.—C.M.L. = chronic myelocytic leukemia; C.L.L. = chronic lymphocytic leukemia. Relationship of serum isomerase to the absolute granulocyte count. The values in chronic myelocytic leukemia are seen to be for the most part below 40 units following continued therapy.

leukemia group there would seem to be parallel increases in the serum isomerase and the absolute granulocyte count of the peripheral blood. Fig. 2 also shows the decrease in serum isomerase in chronic myelocytic leukemia treated with Myleran. This decrease in activity did not always parallel the total leukocyte or granulocyte count. In some patients it occurred within a few weeks ; in others many months elapsed before a change in serum isomerase could be detected.

DISCUSSION

Many cells and tissues, including the leukocytes and erythrocytes, have a high isomerase activity. Bodansky (1954*b*, 1955) has suggested that the rise in serum isomerase in metastatic malignancy may be due to either an excessive production of the enzyme in the new growth or to the easier access into the blood stream of the enzyme from the normal tissues in juxtaposition to the invading tumour. The high levels in chronic myelocytic leukemia may be due to increased production of the enzyme by the leukemic cells or more rapid loss of enzyme from the cells into the serum. The association of high serum isomerase values with the very high granulocyte counts may indicate formation by, or release of, the enzyme from this cell series. The normal values in chronic lymphocytic leukemia suggest that the lymphocytes do not possess or release isomerase to the same degree as do the granulocytes. The serum isomerase does not reflect the total white blood cell count as those cases of chronic lymphocytic leukemia with a high total leukocyte count (up to 295,000/c.mm.) had normal serum isomerase levels. We are now estimating the enzyme in separated leukocytes in an attempt to demonstrate differences in the two series of cells.

Although the total leukocyte count and the granulocyte count were found to be elevated in the infection group, the isomerase was for the most part within normal limits. However, the total granulocyte counts in this group still fell far below those obtained in chronic myelocytic leukemia.

Another possible explanation of the high levels found in chronic myelocytic leukemia might be that bone erosion from the rapidly proliferating myeloid series expanding the medullary cavity facilitates the liberation of isomerase from adjacent tissue. However, the bone marrow invasion and replacement in chronic lymphocytic leukemia is without such an effect on the serum isomerase.

SUMMARY

The serum phosphohexose isomerase acivity was estimated in a normal group and in patients with non-malignant disease, chronic myelocytic and chronic lymphocytic leukemia and leukocytosis due to acute infectious processes. It was found to be elevated in chronic myelocytic leukemia but not in the other groups. In the chronic myelocytic group the isomerase levels fell following treatment.

This work was supported by grants from the National Cancer Institute of Canada and the Manitoba Cancer Institute.

REFERENCES

BODANSKY, O.—(1954*a*), *Cancer*, **7**, 1191.—(1954*b*), *Ibid*, **7**, 1200.—(1955), *Ibid.*, **8**, 1087.

EFFECT OF TUMORS ON LIVER AND SPLEEN UPTAKE OF RADIOACTIVE MATERIAL

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Received for publication April 24, 1956

In the course of recent experiments on possible radioactive chemotherapeutic substances (Argus and Hewson, 1954), we found that fluorene-2,7-di-(sulfonamido-2′-naphthalene)-S^{35} failed to localize in tumor tissue. A reexamination of the data, however, shows that the compound localized in the livers of normal CAF_1/Jax mice to a greater extent than in the livers of CAF_1/Jax mice bearing a transplanted squamous cell stomach carcinoma. This difference was even more pronounced for the spleen. Since such a phenomenon, if general, might be of diagnostic value, it was decided to investigate the following points: could this effect be demonstrated for other transplanted tumors in other strains and species, and what is the pattern of distribution of radioactivity in the liver and spleen?

MATERIALS AND METHODS

A total of sixteen male C3H/Jax mice, three to four months old, were employed. Three to four weeks previous to the study, eight of the animals received a subaxillary transplant of Barrett mammary adenocarcinoma. The tumor weights averaged 688 mg. at the time of sacrifice. Fluorene-2,7-di-(sulfonamido-2′-naphthalene)-S^{35}, having a specific activity of 340,000 dpm/mg., was dissolved in dilute sodium bicarbonate and ethanol, and administered by tail vein; each mouse received 5 mg. of compound. Animals were sacrificed eight hours after injection. The concentration of radioactive material in the tissues was determined by the method previously described (Argus, 1953).

To determine the cells responsible for the uptake of radioactive compound by the liver and spleen, autoradiograms from these organs were made. Frozen sections of the tissue were prepared approximately 10 microns thick. Strips of Kodak, no-screen, X-ray film were exposed to these sections for from one to four weeks. The example shown (Fig. 1) was exposed for one week and developed in D19 for four minutes.

RESULTS AND DISCUSSION

A difference in the uptake per gram of tissue of compound by the spleen of non-tumor as compared to tumor bearing C3H mice was found (Table I). The concentration of radioactivity in the spleen of control mice was 1·8 times that in tumor hosts. Thus the effect found for CAF_1 mouse spleen could also be demonstrated in a different strain with a different tumor.

TABLE I.—*Distribution of Radioactivity at 8 Hours following Intravenous Injection of Fluorene-2,7-di-(sulfonamido-2′-naphthalene)-S^{35}**

Mice.	Tissue.	Average weight of tissue (mg.). Tumor-bearing.	Average weight of tissue (mg.). Control.	Concentration (μg. compound/g. tissue). Tumor-bearing. Range.	Tumor-bearing. Average.†	Control. Range.	Control. Average.†	Concentration ratio. $\left(\frac{\text{Average control}}{\text{Avg. tumor-bearing}}\right)$
C3H	Spleen	202	99	242–304	273	446–556	501	1·8
	Liver	1408	1182	786–1172	979	968–1363	1165	1·2
CAF_1	Spleen	135	78	220–394	307	—	1488	4·8
	Liver	1075	1038	644–760	702	—	1288	1·8

* Dose was 5 mg. compound per mouse.

† Value for CAF_1 control represents 2 pooled animals; each other value represents average from 2 sets of 2 pooled animals.

A concentration ratio of 1·2 was found for the livers of C3H mice. This also may represent a real difference but too much significance cannot be claimed since the concentrations for control and tumor-bearing animals overlap.

In CAF_1 males the concentration in the spleen of non-tumor mice was 4·8 times that in mice bearing the transplanted tumor; the liver of the controls held 1·8 times as much activity as did the spleen from tumor-bearers. Thus we have a larger differential between the non-tumor and tumor-bearing CAF_1 mice than between non-tumor and tumor-bearing C3H mice.

The reason for this variance between the C3H and the CAF_1 mice must be sought in genetic differences between the two. The males of the C3H strain develop spontaneous hepatomas (Agnew and Gardner, 1952). The CAF_1 hybrids are obtained from matings between BALB/c females and A/Jax males. Both of these parent strains are susceptible to spontaneous lung tumors (Andervont, 1938). It would be expected that their offspring would also be susceptible, so that all animals employed can be considered susceptible to spontaneous cancer. Perhaps the difference is in the type of tumor. A proneness to spontaneous liver tumor might well have a more profound effect on the uptake of a compound by the liver, and probably the spleen, than would a propensity to lung cancer. Thus it may be that the difference in concentration ratio in the uptake of the radioactive compound is because the animals are susceptible to different types of spontaneous tumors. On the other hand, two different *transplanted* tumors were employed. Since they both displayed the same degree of malignancy it would not be expected that there would be any great difference in the effect *per se* of a squamous cell stomach carcinoma and a mammary carcinoma on the liver and spleen. The C3H strain, however, does carry the milk factor even though the males do not spontaneously develop mammary cancer. It might well be, therefore, that a transplanted mammary tumor is not as foreign to the C3H male as is a transplanted squamous cell carcinoma to the CAF_1. This could result in the lesser differential found in the uptake of the radioactive compound by the C3H strain.

EXPLANATION OF PLATE

FIG. 1.—Histological section stained with hematoxylin and eosin, and autoradiogram of spleen from a C3H/Jax mouse following administration of fluorene-2,7-di-(sulfonamido-2′-naphthalene)-S^{35}.

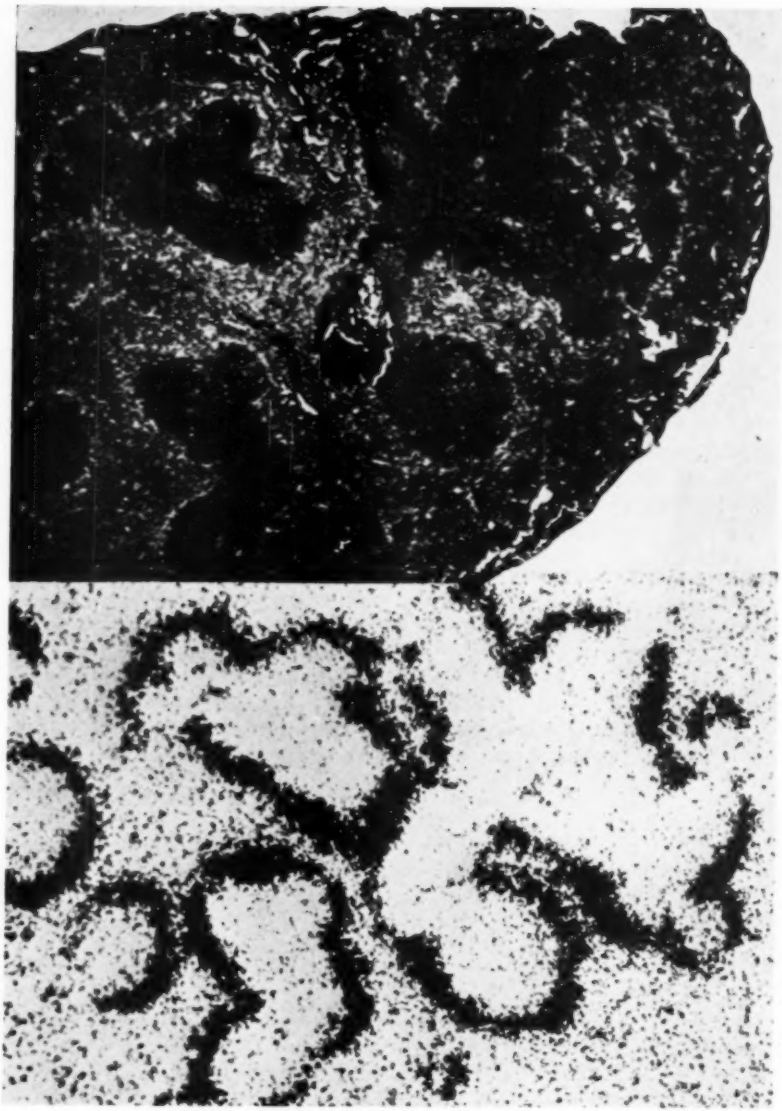

Fig. 1.

Argus, Hewson and Ray.

Autoradiograms.—For the liver, autoradiograms showed a diffuse uptake of radioactive material, suggesting localization in the Kupffer cells. In the spleen (Fig. 1) radioactivity was deposited in the red pulp in a pattern which just outlines the Malpighian follicles. No qualitative difference in this pattern of distribution was seen for the non-tumor and tumor-bearing mice. Dunn (1954*a*, 1954*b*) has described an arrangement of the reticuloendothelial system in the mouse which was demonstrated by Duqué in the C3H strain : surrounding the follicle is a well developed " perifollicular collar " of reticuloendothelial cells one or two layers thick ; peripheral to this there is a " perifollicular halo " formed by reticuloendothelial cells in parallel rows. It appears to be these phagocytic reticulum cells of the " perifollicular halo " that have taken up the radioactivity. This suggests a difference in reticuloendothelial function of tumor-bearing mice as compared to controls.

SUMMARY

Fluorene-2,7-di-(sulfonamido-2′-naphthalene)-S^{35} was administered by tail vein to male C3H/Jax mice. Radioactivity was found to accumulate in the liver and spleen of control mice to a greater extent than in these same organs of mice bearing a transplanted Barrett mammary adenocarcinoma. This difference between non-tumor and tumor-bearing mice of the C3H/Jax strain, however, was not as great as that found between non-tumor and tumor-bearing CAF_1/Jax mice. Autoradiograms of the spleen showed the radioactivity to be deposited in association with phagocytic reticuloendothelial cells outlining the Malpighian follicles.

The present investigation was supported by the American Cancer Society Grant BCH-44 on the recommendation of the Committee on Growth, and by PHS Grant C-1356 from the National Cancer Institute, National Institutes of Health.

The authors are indebted to Dr. Cornelia Hoch-Ligeti and Dr. Thelma Dunn for their analyses of the histological slides and autoradiograms, and to Mrs. Virginia Paul and Miss Hazel Bryant for technical assistance.

REFERENCES

AGNEW, L. R. C. AND GARDNER, W. U.—(1952) *Cancer Res.* **12**, 757.
ANDERVONT, H. B.—(1938) *Publ. Hlth Rep. Wash.*, **53**, 1647.
ARGUS, M. F.—(1953) *Brit. J. Cancer*, **7**, 273.
Idem AND HEWSON, K.—(1954) *Ibid.*, **8**, 698.
DUNN, T. B.—(1954*a*) *J. nat. Cancer Inst.*, **14**, 1281.—(1954*b*) *Ibid.*, **15**, 573.

THE MAMMOTROPHIC POTENCY OF THE URINE OF NORMAL POST-MENOPAUSAL WOMEN

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Received for publication April 30, 1956

AN account of the mammotrophic potency of human urine was recently published in this journal (Hadfield and Young, 1956). The work now presented is a continuation of this enquiry and deals for the most part with the mammotrophic activity of the urine of post-menopausal women.

METHOD.

The urine from 57 post-menopausal women has been examined. The donors were healthy women or hospital in-patients not suffering from any primary endocrinological disorder or from cancer or any other significant disease of the breast, uterus or ovaries. The first specimen of urine passed in the morning after a night's sleep was collected, placed in a refrigerator as soon as possible and kept at about 4° C. during the period of injection. One of us (J. S. Y.) has recently discovered that there is a slow loss of mammotrophic potency from unextracted urines stored at low temperatures. Whenever possible, therefore, the urine should be injected into mice within a week of its receipt as after storage for more than ten days the loss of potency becomes significant.

Each of ten weanling male mice, aged approximately 23 days and weighing between 9 and 11 g., was given 0·2 ml. of the untreated human urine by subcutaneous injection twice daily for five days. The temperature of the urine should be allowed to rise to that of a warm room before injection. We have found these normal urines to be remarkably non-toxic.

The animals were killed and skinned on the sixth morning of the experiment, the skins fixed in Bouin's solution for 24 hours and then transferred to 50 per cent absolute alcohol. Each whole skin was then de-fatted by careful dehydration in absolute alcohol and immersion in several changes of xylol. This operation is carried out in a tissue processor. It very greatly enhances the intensity of staining and facilitates the rapid identification of the glands during dissection. The de-fatted skins are brought back to water and stained in Grenacher's alum carmine.

In each skin an average of five stained mammary glands were identified, dissected out at a magnification of 15 diameters, cleaned of excess fat and muscle, dehydrated and cleared. All the glands from each animal were mounted whole on the same slide without sectioning. It has been a common experience to find that of six mammary glands from one mouse stimulated by a potent urine, two or occasionally three fail to react vigorously and remain small and difficult to identify. It is essential that these small glands should be found and examined. When the urinary potency is low the number of non-reacting glands is far larger

and the final result will be erroneously high if their identification and examination be omitted.

The terminal clubs in each gland were counted. In glands undergoing differentiation and showing replacement of clubs by a cluster of terminal acini, each such acinar mass at a duct end was counted as a club. The total number of clubs in all glands was divided by the total number of glands examined, giving the average clubs per gland. This figure was divided by 1·14, i.e. the average number of clubs per normal gland in the male weanling of the strain we used. This final figure—the mammotrophic "potency"—was regarded as significant if it reached a value of 4 or more. The degree of "acinisation" was determined by enumerating all glands showing clear evidence of glandular differentiation at a magnification of 50 diameters, and expressing this figure as a percentage of all glands examined. Urines in which the mammotrophic potency is high invariably produce a high percentage of acinised glands.

RESULTS

The detailed results of the investigation are shown in Table I.

TABLE I.—*Mammotrophic Potency of Normal Post-menopausal Urine*

Post-menopausal age groups in years.	Total number of donors.	Mammotrophic potency			
		++	+	±	−
0 to 5	15	9·8	6·9	4·4	..
		9·8	6·6	4·2	..
		7·6	6·2	4·1	..
		7·4	5·9	3·5	..
		7·4	5·9	..	..
		7·2	..	..	..
6 to 10	11	12·3	6·6	4·4	2·8
		8·5	4·7	3·6	2·6
		7·2	..	..	1·14
					1·14
11 to 15	14	12·3	6·5	3·85	3·0
		..	5·0	..	2·9
		..	4·7	..	2·1
		..	4·6	..	1·5
					1·1
					0·5
					0·4
					0·35
16 to 20	7	14·5	5·9	4·5	..
		14·2	..	3·6	..
		12·0	..	..	..
		8·0	..	..	..
Over 21	10	8·0	6·4	4·4	3·0
			6·1	4·0	..
			5·6		
			5·0		
			4·9		
			4·6		

DISCUSSION

These results clearly demonstrate that an appreciable proportion of post-menopausal urines possess a significant degree of mammotrophic potency which when present varies from a minimum of 4 to a maximum of 14·5. In analysing

our figures and using as a guide our experience in estimating the potency of pre-menopausal urines, we have classified the results according to the nomenclature shown in Table II.

TABLE II.

Mammotrophic potency.	Designation.
Below 3·4	Absent (−)
Between 3·5 and 4·5	Borderline (±)
Between 4·6 and 7·0	Present (+)
Between 7·1 and 14·5	Present (++)

On this basis the results obtained from urines extending over all post-menopausal age groups (1 to 36 years) may be summarised as indicated in Table III.

TABLE III

Mammotrophic potency.	Percentage of specimens.
Present (+ and ++)	58
Borderline (±)	17·5
Absent (−)	24·5

Table IV shows a further analysis of the 58 per cent of urines having significant degrees of mammotrophic potency.

TABLE IV

Total number of potent urines.	++ Group.	+ Group.
33	15 (i.e. 45·5% of potent urines *or* 26·3% of all urines)	18 (i.e. 54·5% of potent urines *or* 31·5% of all urines)

It is interesting to compare these figures with those obtained from pre-menopausal urines. In doing so the variations in mammotrophic potency during the menstrual cycle must be taken into account. During the first half of the cycle the potency is low; during the second half, between the 17th and 22nd days, it reaches a maximum which varies between 6 and 16·8. A comparable potency range from 6 to 14·5 was found in 25 of the 57 post-menopausal urines examined (i.e. 43·8 per cent). It would therefore appear that the urine of 43·8 per cent of normal post-menopausal women has a mammotrophic potency which fairly closely approximates to that of the normal pre-menopausal woman between the 17th and 22nd days of the cycle.

There is one substantial difference between the pre- and post-menopausal woman in respect of mammotrophic potency. Even during the first half of the cycle, pre-menopausal urine is nearly always potent although the value is low, and very few specimens have no potency. In contrast, potency was completely absent in 24·5 per cent of urines collected over the whole post-menopausal age period, i.e. from 1 to 36 years after cessation of the menses.

Suggestive information is provided by comparing the mammotrophic potency of urines from normal post-menopausal women in all age groups with the results

of cytological examination of vaginal smears and histological examination of the endometrium over the same period. In Table V, vaginae showing clear evidence of oestrogenic stimulation, endometria showing undoubted hyperplasia, and urines showing a significant degree of mammotrophic potency are designated as "positive", whilst vaginae showing no evidence of oestrogenic reaction, atrophic endometria, and urines having no mammotrophic potency are designated as " negative ".

TABLE V.

Examination.	Total number of cases.	Positive (%).	Borderline (%).	Negative (%).
Endometrium*	137	31	24	45
Vaginal cytology†	143	23	19	58
Urine : mammotrophic potency	57	58	17·5	24·5

* = Data of Novak and Richardson (1941).
† = Data provided by J. Stretton Young from an investigation conducted between 1954 and 1956.

The figures for vaginal cytology and mammotrophic potency of post-menopausal urine are compared in the histogram (Fig. 1).

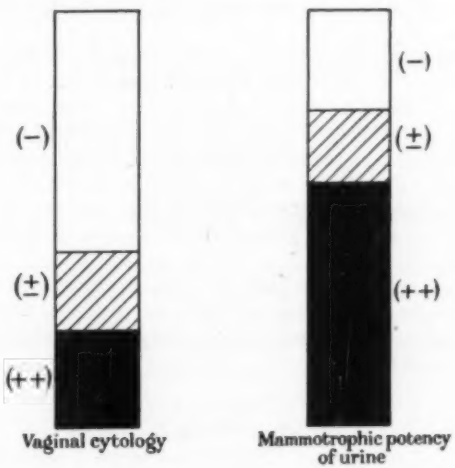

FIG. 1.—Comparison of figures for vaginal cytology and mammotrophic potency of post-menopausal urine.

These results immediately recall the well-established physiological castration phenomenon which occurs in a considerable proportion of post-menopausal women. Ovarian function fails, the blood oestrogen falls and a low blood oestrogen being the appropriate physiological stimulus for the production of gonadotrophin by the hypophysis, the output of this hormone rises and remains high as long as the oestrogen remains low. In Table V 83 of 143 normal post-menopausal women, i.e. 58 per cent, showed no evidence of oestrogenic stimulation of the vaginal

epithelium and there is little doubt that their urinary output of pituitary gonadotrophin was proportionately high. In other words, this well established inverse relationship between a fall in oestrogen production by the ovary and a rise in gonadotrophin production by the hypophysis is enough to make the following safe prediction :

	Positive (%).	Borderline (%).	Negative (%).
Oestrogen response in vaginal epithelium	23	19	58
High urinary output of gonadotrophin	58	19	23

As our figures for the mammotrophic potency of the urine are almost identical with the predicted figures for pituitary gonadtrophin, it is reasonable to suppose that the mammotrophic potency of post-menopausal urine is, in part at least, due to the liberation of a mammotrophic pituitary hormone whose production in post-menopausal women is governed by the same reciprocal relationship with failure of ovarian function as that of gonadotrophin. This supposition obviously needs the confirmation which can only be provided by the simultaneous estimation of the vaginal response, the urinary output of a mammotrophic hormone or hormones and of gonadotrophin in each of a larger series of post-menopausal women than we have been able to examine. The agreement between the two sets of figures also suggests that our numerical criteria for deciding whether a specimen of urine should be regarded as possessing mammotrophic potency or not are reasonably accurate.

When urinary potencies were placed in five-year post-menopausal age groups their distribution from the 16th year onwards was found to be too irregular for satisfactory analysis. This is clearly due to the small number of specimens examined. Analysis of the three periods 0 to 5, 6 to 10 and 11 to 15 years was more satisfactory and showed a significant general tendency for the potency to fall with advancing years. This trend is emphasized if urines showing weak potency or giving an indeterminate result are excluded. Fig. 2 shows the fall in the percentage of urines having considerable potency and an associated rise in those in which potency was absent.

On the other hand, reference to Table I in which our results are shown in detail, will demonstrate that in all the older age groups up to the 29th post-menopausal year there are individuals whose urinary potency is at a high level and examples of these are shown in Table VI.

TABLE VI

Post-menopausal age.	Mammotrophic potency.
15	12·3
16	14·5
16	14·2
20	12·0
20	8·0
24	6·4
26	6·1
29	8·0

CONCLUSIONS

1. The mammotrophic potency of 57 normal post-menopausal urines has been estimated, ten weanling male mice each yielding approximately 50 mammary glands being used for each estimation.

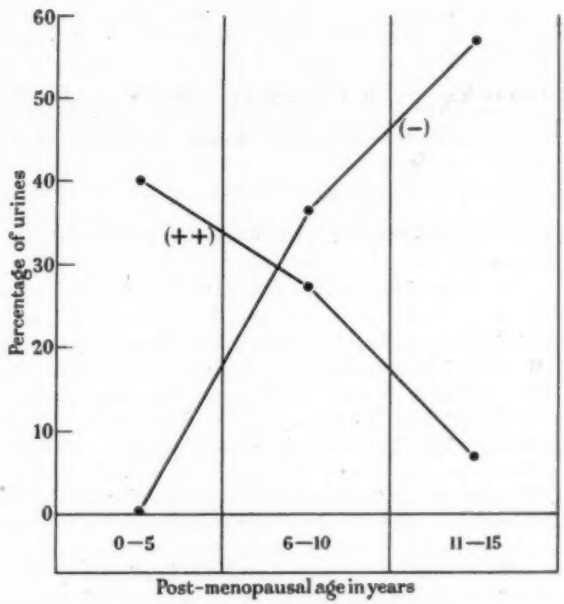

FIG. 2.—Mammotrophic potency of urine in three post-menopausal age groups. Curve labelled (++) shows percentage of urines in each age period having a potency between 7·1 and 14·5. Curve labelled (−) shows percentage in which potency was *nil*.

2. 58 per cent of all urines possessed a significant degree of mammotrophic potency.

3. The excretion of a mammotrophic hormone in the urine of post-menopausal women appears to be related to suppression of ovarian function as its output probably runs parallel with that of pituitary gonadotrophin.

4. Although there was a general tendency for mammotrophic potency to fall with advancing years, it was retained into old age in five individuals between the ages of 70 and 80.

REFERENCES

HADFIELD, G. AND YOUNG, J. STRETTON.—(1956) *Brit. J. Cancer*, **10**, 145.
NOVAK, E. AND RICHARDSON, E. H. Jnr.—(1941) *Amer. J. Obstet. Gynec.*, **42**, 564.

ADDENDUM

It has been recently discovered that in some strains of albino mice the mammary glands of the weanling males do not react to injections of human urine or to prolactin. It is obvious, therefore, that before carrying out an estimation of the mammotrophic potency of human urine, a group of male weanlings of the strain of mice it is proposed to use should be tested for reactivity by injecting the urine of a normal pre-menopausal woman collected between the 18th and 23rd days of the menstrual cycle.

MISCELLANEOUS CHEMICAL CARCINOGENS: CHEMICAL CONSTITUTION AND CARCINOGENIC ACTIVITY

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Received for publication March 6, 1956

The carcinogenic hydrocarbons have been very extensively studied from several different points of view. Many problems still await solution, but the relationship between chemical constitution and carcinogenic activity is becoming clearer (Badger, 1948; Pullman and Pullman, 1955). The azo-compounds have also received considerable attention, and here again some relationship between structure and activity is discernible (Badger, and Lewis, 1952; Pullman and Pullman, 1955). Examples of chemical carcinogens belonging to other classes of compound are also known, and in recent years more attention has been devoted to these substances. Certain aromatic amines are carcinogenic as well as some urethanes, chloro-compounds, "mustards", ethyleneimines, *Senecio* alkaloids, steroids, and some inorganic compounds. The purpose of the present paper is to review the present position with these miscellaneous chemical carcinogens. Radioactive carcinogens and hormonal carcinogens (oestrogens, etc.) are not included.

The diversity of the different types of chemical carcinogen may occasion some surprise. On the other hand, different chemical types are well known to produce many other biological responses. Antibacterial drugs, for example, vary widely in their chemical type, and in their mode of action. In this case it is clearly unprofitable to attempt to find structural relationships between compounds acting by entirely different mechanisms; but the study of the relationship between chemical constitution and antibacterial activity among any one group (say the sulphonamides) may be useful and valuable. In the same way the different types of chemical carcinogens probably have different modes of action. In these circumstances no structural relationship between the polycyclic aromatic hydrocarbons, the ethyleneimines, urethane and carbon tetrachloride, should be looked for. Nevertheless, the study of carcinogenic activity in a series of related aromatic amines may produce information of some importance, as may a similar study of a series of related ethyleneimines, and so on. It should be noted, however, that an observed structural relationship does not necessarily establish that two compounds produce the same biological action by the same mechanism. Most of the sulphonamides probably do have the same mode of action, but in spite of its structural similarity, *p*-sulphonamidobenzylamine is known to differ from sulphanilamide. Furthermore, among the carcinogenic azo-compounds, evidence is accumulating that compounds such as 1-phenylazo-2-naphthol and 2 : 2′-azonaphthalene should be clearly distinguished from the *amino*-azo-compounds such as 4-dimethylaminoazobenzene (Badger, Lewis and Reid, 1954).

INORGANIC CARCINOGENS

Arsenic

For many years the prolonged exposure to small quantities of arsenic, either in the drinking water, by industrial exposure, or by treatment with arsenical drugs, has been thought to be a contributing factor in certain varieties of cancer (Hueper, 1942, 1954). Much of the early evidence was clearly unsatisfactory, and it is still incomplete, but the general consensus of opinion now seems to be that arsenic must be classed as an exogenous carcinogen having a low order of potency (see however Snegireff and Lombard, 1951). A few cases of occupational arsenical cancer have been noted in factories manufacturing sheep-dip preparations (sodium arsenite, and arsenious acid) and insecticides (copper arsenite, calcium arsenate, or lead arsenate), and a very few in the mining and smelting industry. The literature has been carefully reviewed by Neubauer (1947).

A number of cases of " medicinal " arsenical cancer have also been reported in patients treated with various inorganic arsenical drugs such as potassium arsenite and arsenic trioxide. Neubauer collected 143 such cases from the literature, but more have since been reported (e.g. Arhelger and Kremen, 1951 ; Robertson and Clement, 1948 ; Putman, 1945).

The most important trivalent arsenical drugs are the arsphenamines, used for the treatment of syphilis, and Neubauer concludes that these play only an insignificant part in the causation of cancer.

There have been several attempts to produce arsenical cancers in laboratory animals, but no satisfactory evidence of success has been produced. Part of the difficulty is probably associated with the toxic nature of the material ; otherwise it must be tentatively assumed that man is more susceptible to arsenic than the laboratory animals used so far.

Beryllium

The production of osteogenic sarcomas in rabbits following the injection of zinc beryllium silicate and beryllium oxide was first noted by Gardner and Heslington (1946), and has since been confirmed by many other workers (Barnes, 1950 ; Nash, 1950 ; Hoagland, Grier and Hood, 1950 ; Dutra and Largent, 1950 ; Barnes, Denz and Sissons, 1950, Sissons, 1950).

An osteogenic sarcoma has been reported following the inhalation of beryllium oxide (Dutra, Largent and Roth, 1951) and finely powdered beryllium metal has also been shown to be effective when administered by intravenous injection as an aqueous suspension (Barnes, 1950).

This experimental production of tumours using beryllium and beryllium compounds is of considerable importance in view of the industrial use of these materials. Among workers in the industry, pulmonary reactions and skin lesions appear to be common, and subcutaneous granuloma have been reported in persons who cut themselves on fluorescent lamps containing beryllium (Van Ordstrand, Hughes, DeNardi and Carmody, 1945 ; Grier, Nash and Freiman, 1948).

Chromium

Several workers have reported that the incidence of cancer of the lung is significantly higher among workers in the chromate industry than in comparable groups not exposed to compounds of this metal (Machle and Gregorius, 1948 ;

Baetjer, 1950; Bourne and Yee, 1950; Bidstrup, 1950, 1951; Mancuso and Hueper, 1951). It would seem therefore that chromium must be added to the list of inorganic carcinogens and further experimental work to determine the exact nature of the carcinogen is urgently required.

Cobalt

Tumours at the site of injection have been produced following the administration of pure 400-mesh cobalt powder (mixed with fowl serum) to rats of the hooded strain (Heath, 1954).

Nickel

Hueper (1952) has reported the production of a number of cancers in rats following the injection of nickel powder in lanolin. Most of the tumours were apparently osteogenic sarcomas, but one originated from connective tissue at the site of injection, one from the epithelial lining of an osteomyelitic fistula, and two seemed to have developed from abdominal lymph nodes. The minimum latent period was about six months.

It may be noted that men working in the Mond nickel process show a high incidence of cancer of the lung and of the nasal passages (Bidstrup, 1950).

Zinc

Zinc chloride has been found to produce teratoma of the testis in a small percentage of cases following injection into the testes of adult roosters (Bagg, 1936; Anissimova, 1939; Michalowsky, 1928). Zinc sulphate behaves similarly (Falin and Gromzewa, 1939).

General survey of inorganic carcinogens

Tumours have sometimes been attributed to the non-specific action of such substances as hydrochloric acid and sodium hydroxide (Narat, 1925; Suntzeff, Babcock and Loeb, 1940). Tumours have also been produced following the administration of various radioactive substances, and it is generally assumed (but without adequate evidence) that the carcinogenic action is due to the radiations emitted. For example, the injection of powdered metallic uranium (a pure alpha-ray emitter) into 33 rats resulted in the development of 11 sarcomas at the site of injection; but it is not known whether this is a metallo-carcinogenic effect or a radio-carcinogenic effect (Hueper, Zuefle, Link and Johnson, 1952).

As far as can be judged, however, the carcinogenic action of the inorganic substances mentioned in the sections above is a specific one; but there seems to be no knowledge of their mode of action. As a working hypothesis, however, it may be worth noting that (with the exception of beryllium, the carcinogenic activity of which is highly specialised) all these elements would be expected to combine with sulphur groups in certain enzymes and proteins, etc., and such an interference might well initiate the carcinogenic process. In this respect, therefore, the inorganic ions may well resemble the carcinogenic hydrocarbons in their mode of action. The reactivity of various metal ions towards sulphur groups is reflected in their action towards substances such as BAL, penicillin, etc. For example, BAL is effective against arsenic, mercury, nickel, cadmium, chromium, antimony

and some others (Thompson and Whittaker, 1947 ; Graham and hood, 1948 ; Russell, Green and Wand, 1948).

The present list of inorganic carcinogens is unlikely to be complete, and indeed there is a brief reference to the possible carcinogenic activity of tin (Larionov, 1930). Clearly further work is required in this field.

AROMATIC AMINES

"*Aniline cancer*"

In 1895 Rehn reported three cases of bladder tumour occurring among men engaged in the manufacture of fuchsine (magenta). As the number of workmen involved was small, he thought this was an undue incidence and he regarded the tumours as of occupational origin. The aniline used in the manufacturing process was suggested as the most probable carcinogen.

Further cases of bladder tumours occurring in dyestuffs operatives were soon reported by other workers. Hueper (1938) estimated the total number of recorded cases of this type of occupational cancer as 550 ; but the actual number was doubtless greater than this. More recently, Case, Hosker, McDonald and Pearson (1954) have stated that 455 cases of bladder tumour have been found in the British chemical industry up to February 1, 1952 ; of these, 444 were found in and after 1921, the first year in which death certificates were available for systematic search.

In many of the early investigations aniline often seemed to be implicated, and this form of occupational cancer came to be known as " aniline cancer ". Within the last few years, however, a thorough statistical examination has been completed, and no evidence was found that aniline causes an increased number of bladder tumours in men who manufacture or handle it (Case, Hosker, McDonald and Pearson, 1954). On the other hand, it must be remembered that the aniline used in the chemical industry today is a fairly pure product, quite unlike the crude material commonly used in the 19th century and at the beginning of the 20th century. The aniline used today seems to be non-carcinogenic ; but it is possible that the crude aniline of the last century may have contained a carcinogenic impurity, possibly 4-aminodiphenyl (Walpole, Williams and Roberts, 1952).

The original observations of Rehn referred to the manufacture of fuchsine (magenta) ; but workers engaged in the manufacture of other dyestuffs and intermediates have also been shown to be especially liable to cancer of the bladder. There have been many attempts to identify the carcinogens involved and until recently there have been some differences of opinion on the matter.

Recent statistical surveys have demonstrated that contact with benzidine, 1-naphthylamine and 2-naphthylamine, in either manufacture or use, can cause bladder tumours (Case, Hoskar, McDonald and Pearson, 1954 ; Case and Pearson, 1954 ; Goldblatt, 1949 ; Scott, 1952 ; Barsotti and Vigliani, 1952). It seems that 2-naphthylamine has been the most potent cause of occupational bladder tumours between 1915 and 1951, the average induction period being 16 years. A similar average induction period was found for benzidine, that for 1-naphthylamine being 22 years. Among certain groups of men the hazard is very high and one in five may expect to develop bladder tumours. The manufacture of 2-naphthylamine has accordingly been discontinued by many of the major chemical companies.

The manufacture of fuchsine (magenta) and of auramine also produces an occupational hazard of the same nature, but the available data do not indicate whether an intermediate or the final product is involved in tumour production.

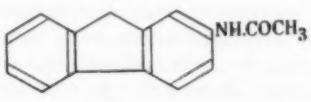
For many years all attempts to confirm the carcinogenic activity of various aromatic amines by experimental tests in laboratory animals were without positive result (see, for example, Berenblum and Bonser, 1937). In some cases the experiments were of insufficient duration ; in others, non-susceptible animals were used.

In 1938, however, Hueper, Wiley and Wolfe obtained bladder tumours in dogs following administration of commercial 2-naphthylamine. A few years later Bonser (1943) obtained epithelial tumours of the bladder in dogs following massive oral administration of a purified 2-naphthylamine over a period of five years. Bladder tumours have also been obtained in mice, rats and in rabbits, but these species are far less susceptible, and in the case of rats and rabbits only benign tumours were obtained (Bonser, Clayson, Jull and Pyrah, 1952 ; Bonser, Clayson and Jull, 1951). Experimental bladder tumours have not been induced with 1-naphthylamine. Benzidine, however, has been shown to produce bladder tumours (with difficulty) in the dog, and it has induced tumours at a number of sites following administration to rats (Spitz, Maguigan and Dobriner, 1950; Walpole, Williams and Roberts, 1954).

Detailed examination of aromatic amines

The aromatic amines have not been as extensively investigated as the polycyclic hydrocarbons, but it is already clear that many compounds of this nature possess carcinogenic activity.

Although dyestuffs-intermediates are of prime interest in this field, much of the recent work has stemmed from the discovery that the insecticide 2-acetylaminofluorene (I) is a potent carcinogen (Wilson, DeEds and Cox, 1941). When incorporated into the diet of rats it produced multiple tumours including those of the liver, bladder, breast and auditory canal. The minimum effective concentration in the diet was found to be 0·004 per cent, the maximum tolerated being 0·125 per cent. The latter dose produced tumours in a very short time, but the mortality was high. A daily dose of 4 mg. given for 25 weeks was found to produce tumours in more than 90 per cent of the animals in 42 weeks or less (Bielschowsky, 1944a, 1947 ; Dunning, Curtis and Madsen, 1947).



I

The distribution of tumours in the various organs has been shown to vary considerably with the sex and strain of the rat under test (Harris, 1947), and the distribution can also be affected by other factors. Bielschowsky (1944*b*) found that although tumours of the thyroid are not produced by 2-acetylaminofluorene alone, such neoplasms are common when it is administered together with a goitrogenic substance such as allyl thiourea, or methyl thiouracil (Hall, 1948). Similarly, thyroid tumours were produced by feeding 2-acetylaminofluorene for 13-25 weeks to partially thyroidectomized rats (Bielschowsky, 1949). Complete

thyroidectomy prior to the administration of 2-acetylaminofluorene prevents the development of neoplasms of the liver; but tumours were observed in several other organs, the most frequently affected site being the *meatus acousticus externus* (Bielschowsky and Hall, 1953). (It should be noted that thyroid adenomata have also been observed following prolonged administration of goitrogenic substances (allyl thiourea and thiourea) alone (Bielschowsky, 1945a; Purves and Griesbach, 1947), and the prolonged administration of thiourea and of thioacetamide is also reported to produce liver tumours (Fitzhugh and Nelson, 1948; Dupta, 1955).)

The influence of sex hormones on the distribution of tumours has also been studied. The incidence of mammary carcinoma was not increased by the simultaneous administration of 2-acetylaminofluorene and oestrogen, but it was increased enormously when progesterone was substituted for the oestrogen (Kirby, 1947; Cantarow, Stasney and Paschkis, 1948).

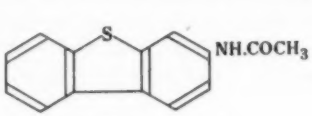
2-Acetylaminofluorene has also been shown to be a carcinogen when given to several different strains of mice, but this species was found to be more resistant than the rat (Armstrong and Bonser, 1944, 1947; Foulds, 1947; Wilson, DeEds and Cox, 1947*a*). Here again strain differences were found to be important. For example, of five strains of mice tested, the CBA strain showed the greatest susceptibility to bladder tumours.

Some interesting metabolic studies have been reported using 2-acetylaminofluorene labelled with C^{14} in the terminal carbon atom of the acetyl group. With this compound, 6 per cent of the administered radioactivity was eliminated in the breath of rats within six hours. On the other hand there was a negligible amount of activity in the breath following administration of the 9–C^{14} labelled compound. It seems likely that part at least of the administered 2-acetylaminofluorene may be hydrolysed *in vivo*, the acetyl residue being metabolised independently of the remainder of the molecule (Morris, Weisburger and Weisburger, 1950). It is not surprising, therefore, that the parent amine, 2-aminofluorene, also produced multiple tumours in both rats and mice receiving it in the diet; but it does seem to be less effective than the acetyl derivative (Wilson, DeEds and Cox, 1947*b*). When administered to rats by skin-painting in acetone solution, all the animals developed liver tumours by 280 days, and one had a tumour of the *ductus acusticus externus*; but no skin cancers were produced (Bielschowsky, 1944*a*). With mice, only one tumour appeared at the site of painting in a total of 28 mice surviving more than 200 days (Kirby, 1948). Mice receiving the parent amine by injection showed a low incidence of tumours at the site of injection, in the liver, bladder, kidney, mammary gland and ovary.

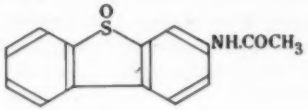
Several closely related compounds have also been tested, including 2-diacetylaminofluorene 2-monomethylaminofluorene, 2-dimethylaminofluorene and 7-fluoro-2-acetylaminofluorene. All these compounds are active, but they are less active than 2-acetylaminofluorene, and in general they are less effective in producing multiple tumours. 2-Acetylamino-7-hydroxyfluorene, which is a metabolite of 2-acetylaminofluorene, proved to be inactive (Bielschowsky, 1954*b*, 1947). Fluorene itself is also inactive, but 2-nitrofluorene is active and it seems likely that it is reduced *in vivo* to 2-aminofluorene.

Other closely related compounds include the heterocyclic derivatives 3-acetylaminodibenzothiophen (II), 3-acetylaminodibenzothiophen-5-oxide (III), and 3-acetylaminodibenzofuran (IV). None of these compounds produced cancers of

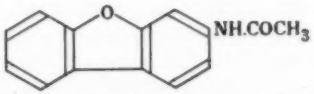
the liver, but 3-acetylaminodibenzothiophen (II) was found to be as effective as 2-acetylaminofluorene in inducing cancers in the mammary gland and in ear duct tissue. The remaining two compounds (III and IV) proved somewhat less effective in this respect (Miller, Miller, Sandin, and Brown, 1949).



II



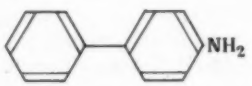
III



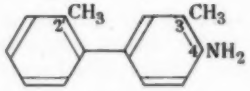
IV

These differences in the distribution of the tumours are unlikely to be of fundamental importance. As Clayson (1953) has pointed out, the widespread distribution of tumours produced by compounds of the acetylaminofluorene type suggests that the active agents circulate and induce tumours where their concentration and other factors are favourable. Small differences in chemical structure can easily affect the way in which the substance circulates. Moreover, as mentioned above 2-acetylaminofluorene produces tumours of the thyroid of the rat only when this gland is stimulated by a goitrogenic agent or by other means.

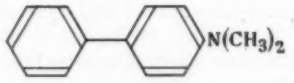
In these compounds, the $-CH_2-$ bridge which occurs in 2-acetylaminofluorene has been replaced by a hetero-atom ; but a bridge is not essential for carcinogenic activity. 4-Aminodiphenyl (V) has been shown to be a potent carcinogen in the rat when administered by subcutaneous injection, producing tumours at a variety of sites (Walpole, Williams and Roberts, 1952). Moreover, it produces bladder tumours in dogs receiving the material by mouth (Walpole, Williams and Roberts, 1954, 1955). Similarly, 3 : 2′-dimethyl-4-aminodiphenyl (VI) has been found to be even more potent than 4-aminodiphenyl in the rat (Walpole, Williams and Roberts, 1952) ; and 4-dimethylaminodiphenyl (VII) produced tumours in the mammary glands, ear duct, liver and vertebral canal of male rats (Miller, Miller, Sandin and Brown, 1949).



V



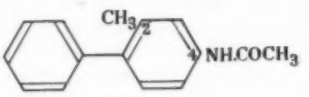
VI



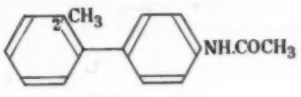
VII

It is of some interest that 4-acetylaminodiphenyl produces tumours in the mammary gland and ear duct in female rats, but is inactive in males. 2-Methyl-

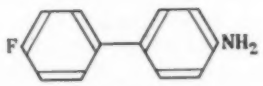
4-acetylaminodiphenyl (VIII) and 2′-methyl-4-acetylaminodiphenyl (IX) are inactive in rats of both sexes (Sandin, Melby, Hay, Jones, Miller and Miller, 1952).



VIII



IX

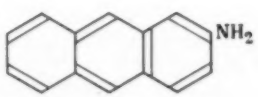


X

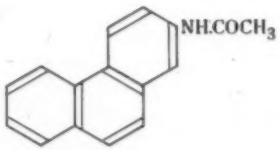
As 4-aminodiphenyl is largely metabolised to the 4′-hydroxy derivative, the testing of a derivative in which the 4′-position is blocked was clearly of interest. Hendry, Matthews, Walpole and Williams (1955) therefore examined 4′-fluoro-4-aminodiphenyl (X) and found it to be more active than the parent amine.

However, the introduction of the fluoro-substituent also produced a profound change in the pattern of tumour incidence. With 4-aminodiphenyl itself the commonest tumours were intestinal; but with the 4′-fluoro- derivative, tumours appeared predominantly in the liver and kidney.

Aromatic amines of some other types are also cancer producing. 2-Aminoanthracene (XI) is closely related to 2-aminofluorene, and it has produced liver tumours in mice receiving the compound by subcutaneous injection (Shear, 1938). Tumours of the skin have also been produced in rats following prolonged skin-painting with an acetone solution (Bielschowsky, 1946).



XI

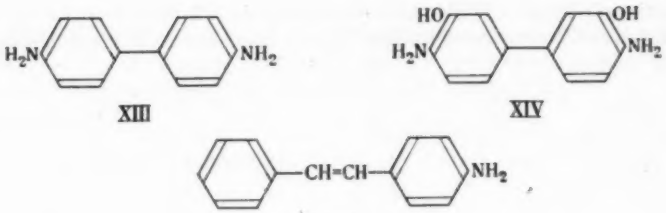


XII

2-Acetylaminophenanthrene (XII) has produced tumours of the mammary gland, ear duct, and intestinal epithelium, and also showed a moderate leukemogenic activity. 3-Acetylaminophenanthrene also produced tumours of the mammary gland, ear duct and small intestine (Miller, Sandin, Miller and Rusch, 1955).

All these amines are, of course, also related to benzidine (XIII), which has been proved to be a bladder carcinogen for man, which produces bladder tumours in the dog, and which gives a variety of tumours in the rat (Spitz, Maguigan and Dobriner, 1950; Walpole; Williams and Roberts, 1954). 3 : 3′-Dihydroxybenzidine (XIV), a probable metabolite of benzidine, is also carcinogenic to rats and mice (Baker, 1950, 1953).

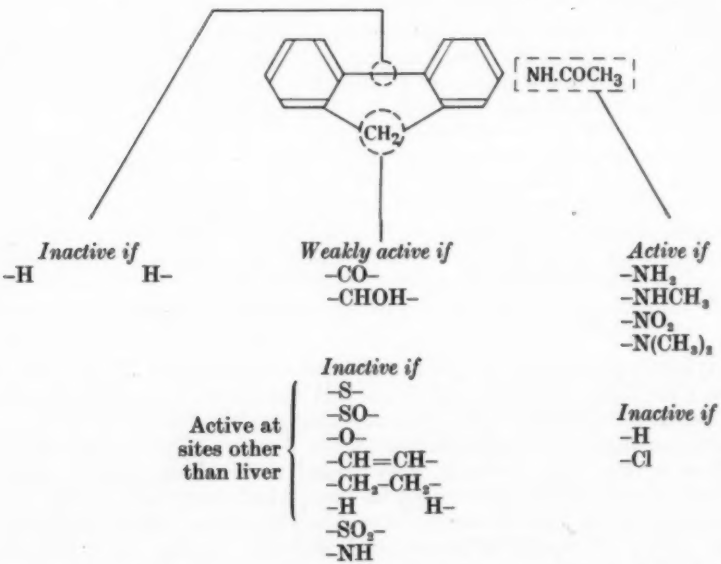
The aminostilbenes represent yet another type of carcinogenic amine. Relatively few members of this group have been tested for carcinogenic activity, having been chiefly studied for their growth-inhibitory activity. However,



XIII XIV XV

4-aminostilbene (XV), 4-dimethylaminostilbene, 4-dimethylamino-2′-methylstilbene and 1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)ethylene have been found to produce a wide variety of tumours in rats when administered by subcutaneous injection. In mice the compounds were found to be far less active (Haddow, Harris, Kon and Roe, 1948).

To conclude this section, it seems that although the carcinogenic aromatic amines are of rather diverse structure, the requirements for activity are becoming clearer. For example, among the derivatives and analogues of 2-acetylaminofluorene the structural requirements for activity against the liver are summarised in the following chart which is due to Miller, Sandin, Miller and Rusch (1955).



This summary would appear to indicate that a fluorene nucleus is essential for strong carcinogenic activity towards the liver ; but if other structural features are *also* changed, this would not seem to be true. 4′-Fluoro-4-aminodiphenyl does produce tumours of the liver, and in this connection it is noteworthy that

7-fluoro-2-acetylaminofluorene is much more active towards the liver of both male and female rats than 2-acetylaminofluorene. It is reasonable to conclude therefore that certain structural features may profoundly affect the distribution of the compound in the animal body and hence the distribution of tumours. On the other hand, these same structural features may have little or no effect in governing whether a certain substance is actually carcinogenic or not.

It has been suggested that the greater carcinogenic activity of derivatives of fluorene *versus* diphenyl derivatives is associated with the $-CH_2-$ bridge which helps to maintain a coplanar arrangement of the benzene nuclei (Miller, Miller, Sandin and Brown, 1949; Sandin *et al.*, 1952). In this connection attention has been called to the striking difference in the incidence and distribution of tumours produced by 2-acetylaminofluorene, and 4-acetylaminodiphenyl, and the lack of activity in 2-methyl-4-acetylaminodiphenyl and 2′-methyl-4-acetylaminodiphenyl (Table I). Spectrographic evidence confirms the suggestion that planarity is greater in 2-acetylaminofluorene than in 4-acetylaminodiphenyl, and it also indicates that the two methyl derivatives are less planar than 4-acetylaminodiphenyl itself.

TABLE I.*—*Carcinogenic Activities of 2-Acetylaminofluorene and Certain Diphenyl Derivatives.*†

Compound.	Sex.	Number of rats studied.	Number of rats with tumours in			
			Liver.	Mammary gland.	Ear duct.	Small intestine.
2-Acetylaminofluorene	M	26	22	0	10	13
	F	25	0	21	20	5
4-Acetylaminodiphenyl	M	15	0	0	0	0
	F	15	0	11	2	0
2-Methyl-4-acetylaminodiphenyl	M	9	0	0	0	0
	F	9	0	0	0	0
2′-Methyl-4-acetylaminodiphenyl	M	9	0	0	0	0
	F	9	0	0	0	0

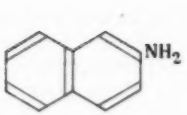
* Sandin *et al.* (1952).

† Final tumour incidences in rats fed 1·62 m. moles of compound per kg. of a grain diet for 8 months and the grain diet alone for an additional 2 months.

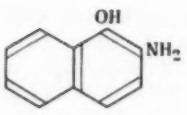
Nevertheless planarity cannot be a sole determining factor for pronounced activity, and in particular for activity against the liver, for the methyl derivative, 3 : 2′-dimethyl-4-aminodiphenyl, in which there is just as much restriction to planarity, is more active than 4-aminodiphenyl itself. Moreover, as mentioned above, the introduction of a fluoro-substituent seems to increase the activity, particularly towards the liver, and this substitution can have little effect on the planarity of the molecule.

Metabolism of aromatic amines

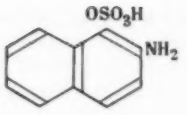
When 2-naphthylamine (XVI) is administered to dogs it is oxidised to 2-amino-1-naphthol (XVII) and excreted as ethereal sulphate (XVIII) (Wiley, 1938; Bonser, Clayson and Jull, 1951).



XVI



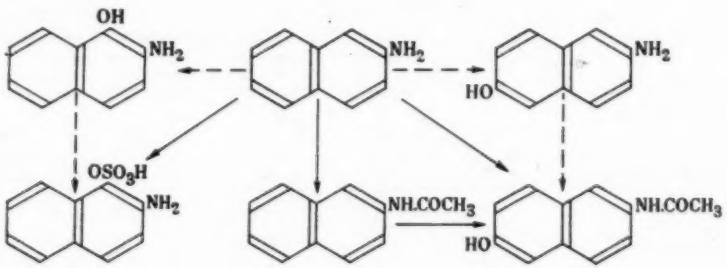
XVII



XVIII

In rats, rabbits and monkeys, however, the administration of 2-naphthylamine by subcutaneous injection was found to be followed by the excretion in the urine of 2-acetylaminonaphthalene and 2-acetylamino-6-hydroxynaphthalene, together with unchanged amine (Dobriner, Hofmann and Rhoads, 1941). Subsequently, it has been found that some 2-naphthylamine-1-sulphuric acid is also excreted by rats. It has also been found that administration of 2-acetylaminonaphthalene to rats by subcutaneous injection, is followed by the excretion of 2-acetylamino-6-hydroxynaphthalene (Manson and Young, 1950).

These metabolic transformations of 2-naphthylamine in the rat are summarised in the following chart in which conversions which have been shown to take place are indicated by solid arrows. Possible intermediary reactions are indicated by broken arrows.



It seems, therefore, that 2-naphthylamine is metabolised by two distinct routes in laboratory animals, one leading to a 2-amino-1-naphthol derivative, and the other to a 2-amino-6-naphthol derivative. Clayson (1950) has developed a method for the estimation of 2-amino-1-naphthol conjugates in the urine, and Bonser, Clayson and Jull (1951) have used the procedure to determine the urinary excretion of the amine in different species. The results are summarised in Table II.

It seems that there is an approximate correlation between the tumour-susceptibility of the species and the amount of 2-amino-1-naphthol conjugate excreted. The dog, which is particularly susceptible to 2-naphthylamine carcinogenesis excretes a high percentage of the dose as 2-amino-1-naphthol conjugates. On the other hand, the mouse, rat and rabbit, which are less susceptible, excrete smaller quantities in this form.

Bonser, Clayson and Jull concluded that 2-amino-1-naphthol is the actual carcinogen and that the parent amine, 2-naphthylamine, is without direct activity. 2-Aminno-1-naphthol hydrochloride was accordingly tested as a local carcinogen using a method involving the surgical introduction of paraffin wax pellets containing the material into the lumen of the bladder of the mouse. Under these conditions 2-amino-1-naphthol hydrochloride proved to be an active carcinogen of the same

TABLE II.—*Excretion of 2-amino-1-naphthol Conjugates in Urine of Various Species**

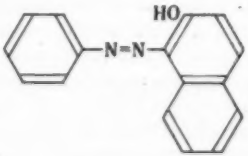
Species.	Dose (mg/kg.).	Route of administration.	Recovery of metabolite (percentage of dose).
Dog	30	By mouth	55–70
	120	„ „	30–45
Cat	50	„ „	30–50
Mouse	120	„ „	20–40
Rat	25	Intraperitoneal	6–9†
	150	„	12–15†
Rabbit	200	By mouth	about 5

* Bonser, Clayson and Jull (1951).

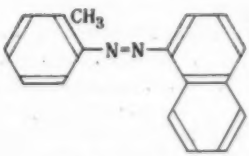
† The lower value was obtained in rats given a high-protein (20 per cent) diet, and the higher value in rats given a low-protein (5 per cent) diet.

order of potency as methylcholanthrene ; but 2-naphthylamine itself proved to be inactive when tested in this way. Moreover, the subcutaneous injection of 2-amino-1-naphthol hydrochloride in oil has given a few sarcomas in mice and rats (Bonser, Clayson, Jull and Pyrah, 1952). (It may be noted that the rat is not a suitable test animal for investigations involving implantation of wax pellets into the bladder. Paraffin wax itself can cause tumours in the bladder of this species (Bonser, Clayson, Jull and Pyrah, 1953).)

Largely on the basis of these results, it has been suggested that all carcinogenic amines may owe their activity to the fact that they are converted, at least in part, to *ortho*-hydroxyamines (Walpole, Williams and Roberts, 1952 ; Clayson, 1953). Certain structural features, as well as the species of animal under test, may affect the proportion of *ortho*-hydroxyamine formed, and may therefore be of prime importance. For example, it has been suggested that those aromatic amines which have a blocked *para* position will tend to hydroxylate to a greater extent in the *ortho*- position, and thus give rise to carcinogenic substances. Furthermore it may well be that 1-phenylazo-2-naphthol (XIX) and 1-*o*-tolylazo-2-naphthol (XX) are reduced *in vivo* to 1-amino-2-naphthol, and this might account for the undoubted carcinogenic activity of these food-colouring-matters in mice (Kirby and Peacock, 1949 ; Bonser, Clayson and Jull, 1954).



XIX



XX

In order to test this general hypothesis, 2-aminophenol, 3-amino-2-naphthol hydrochloride, and 1-amino-2-naphthol-4-sulphonic acid (sodium salt) were tested by the bladder-implantation technique in mice (British Empire Cancer

Campaign, 1953) but were found to be inactive. An impure specimen of 1-amino-2-naphthol hydrochloride did give tumours however, as did 1-phenylazo-2-naphthol and 4-hydroxy-3-aminodiphenyl hydrochloride.

Walpole, Williams and Roberts (1952) pointed out that the metabolic yield of *o*-hydroxyamines is increased by *o*-methylation and by N : N-dimethylation, and they therefore suggested that *o*-toluidine and dimethylaniline, but not aniline, should produce bladder tumours in the dog. However, this suggestion has been considerably weakened by the failure to confirm any local carcinogenic action with *o*-aminophenol. Moreover, Druckney and Schmähl (1954) and Druckney, Schmähl and Reiter (1954) have tested the three isomeric N : N-dimethyltoluidines in rats in doses of up to 10 g. per animal over the entire life span. No chronic toxic effects were observed, the average life span was not diminished and no carcinogenic activity was observed. Finally, 2-hydroxy-4-dimethylaminoazobenzene and 2′-hydroxy-4-dimethylaminoazobenzene, both of which would be expected to give *o*-hydroxyamines on metabolic reduction of the azo- group, have failed to give tumours (Badger, and Lewis, 1952).

Further work is clearly necessary in order to test this interesting hypothesis. In support it may be noted that although 2-amino-7-hydroxyfluorene has been identified as a metabolite of 2-acetylaminofluorene in rats (Bielschowsky, 1945*b*) ; in recent work using carrier techniques, 1-hydroxy-, 3-hydroxy-, and 5-hydroxy-2-acetylaminofluorene have also been identified as metabolites in the urine of rats fed 2-acetylaminofluorene-9-C^{14} (Weisburger and Weisburger, 1954, 1955). The testing of these substances as local carcinogens will therefore be awaited with interest. Similarly 2-hydroxy-3-acetylaminofluorene has been found to be a metabolite of 3-acetylaminofluorene in the rat (Weisburger, 1955).

Furthermore, benzidine seems to be metabolised to 3-hydroxybenzidine and 4 : 4′-diamino-3-diphenylyl hydrogen sulphate in the dog (British Empire Cancer Campaign, 1954, p. 223 ; Bradshaw and Clayson, 1955). In humans it is metabolised, at least in part, to 3 : 3′-dihydroxybenzidine, and when fed to rats this metabolite rapidly produced intestinal tumours (Baker, 1950, 1953 ; Adler, 1908 ; Walpole, Williams and Roberts, 1952). On the other hand, 3 : 3′-dihydroxybenzidine proved inactive when tested by the bladder-implantation technique in in mice (British Empire Cancer Campaign, 1953) so the significance of the former result is in some doubt.

In dogs, 4-aminodiphenyl is metabolised to 4-amino-3-diphenylyl hydrogen sulphate. When the urine was hydrolysed by boiling with acid, 3-hydroxy-4-aminodiphenyl was identified (Bradshaw and Clayson, 1955). All these reports therefore support the view that *ortho*-hydroxylation is of considerable importance in the carcinogenic process.

CHOLESTEROL AND RELATED COMPOUNDS

Although the discovery of 3 : 4-benzopyrene and of other similar carcinogens has done much to explain the incidence of some occupational and environmental cancers, the great majority of human cancers remain " spontaneous " and unexplained. In this connection the hypothesis that a chemical carcinogen may be formed *in vivo* by some abnormal mechanism should clearly be investigated, and work has proceeded along several different lines.

In 1932 Kennaway and Cook suggested that a polycyclic aromatic hydrocarbon

might arise from certain steroids by some abnormal mechanism. Soon afterwards, 20-methylcholanthrene was prepared from deoxycholic acid, albeit by laboratory methods (Cook, 1933 ; Cook and Haslewood, 1933, 1934, 1935 ; Wieland and Dane, 1933), and this 1 : 2-benzanthracene derivative was soon found to be a very potent carcinogen (Barry *et al.*, 1935).

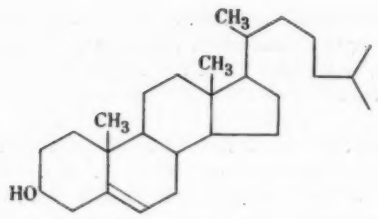
Methylcholanthrene has also been prepared from cholic acid and from cholesterol ; but there is no evidence that transformations of this type do in fact proceed *in vivo*. At the moment, this work must be considered solely as an interesting speculation. There is, however, some evidence that deoxycholic acid itself is carcinogenic when given to mice by injection in sesame oil (Badger, Cook, Hewett, Kennaway, Kennaway and Martin, 1942).

Another suggestion has been that polycyclic aromatic hydrocarbons might arise from steroids, etc., during the cooking of food, and this hypothesis has also been examined (Peacock, 1947). A carcinogenic tar has certainly been produced by the pyrolysis of cholesterol (Kennaway and Sampson, 1928), but the temperature involved was much greater than could occur in cooking. More recently, Falk, Goldfein and Steiner (1949) have studied the pyrolysis of cholesterol at 360°, and the following compounds were identified : cholestenone, dicholesteryl ether, 3 : 5-cholestadiene, the naphthalene derivative of cholesterol, the phenanthrene derivative of cholesterol, methylcyclopentenophenanthrene and chrysene. Except for cholestenone, all these products may be considered as intermediates in the transformation of cholesterol to methylcholanthrene ; but the latter compound was not found.

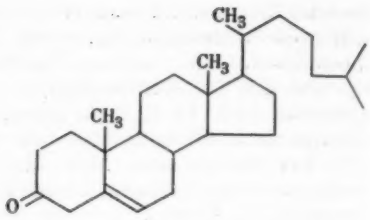
An investigation of a different kind was initiated by Hieger (1946, 1947, 1949) who has shown that cholesterol (XXI) has definite if weak carcinogenic activity. The first experiments were carried out using a commercial cholesterol, which gave 25 sarcomas in 436 mice. Subsequently, however, it was found that purified cholesterol is also cancer-producing (Hieger and Orr, 1954).

Fieser (1954) has pointed out that as the total quantity of cholesterol in a man weighing 65 kg. is approximately 210 g., or 0·3 per cent of the wet weight, it is unlikely that it can have the properties of a carcinogen. He suggested that the observed activity must be due to a transformation product. In this connection it is noteworthy that a crude progesterone preparation, prepared by permanganate oxidation of cholesterol dibromide, and debromination, produced tumours in 32 per cent of the mice tested (Bischoff and Rupp, 1946 ; Spielman and Meyer, 1939).

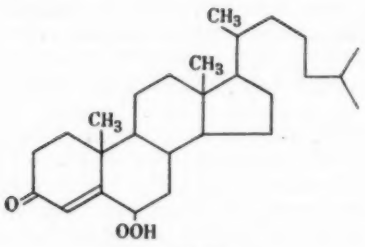
Reasoning in terms of the chemical reactivity required for carcinogenic activity, Fieser (1954) suggested that Δ^5-cholestene-3-one (XXII) might be the true carcinogen formed from cholesterol. It has recently been found (Fieser, Greene, Bischoff, Lopez and Rupp, 1955) that the hydroperoxide (XXIII) derived from this compound is an active carcinogen. This hydroperoxide, 6 β-hydroperoxy-Δ^4-cholestene-3-one (XXIII), was given by subcutaneous injection to 32 mice, and at the age of 12 months fibrosarcomas had appeared at the site of injection in 13 of the mice treated (average tumour age, 9·6 months) and 17 of the remaining mice were still alive. Fieser's views on the chemical reactivity requirements for carcinogenic activity have therefore been supported in a very satisfactory way, and further work in this field will be awaited with interest. It may be added that there is some indirect evidence that Δ^5-cholestene-3-one can be formed from cholesterol in the body.



XXI



XXII



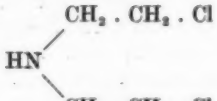
XXIII

ALKYLATING AGENTS

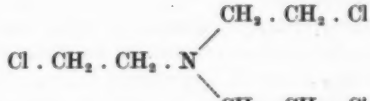
Nitrogen and sulphur mustards

Various war-time studies showed that the nitrogen and sulphur mustards resemble ionizing radiations in many of their biological effects, particularly in regard to the inhibition of cell division and growth, the production of mutations and chromosome damage. Compounds of this nature have accordingly been extensively investigated as it was hoped that an effective tumour-inhibitor might be found. A few have also been tested for carcinogenic activity.

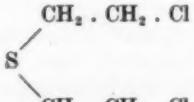
The nitrogen mustards do not seem to be carcinogenic to the skin of mice (Fell and Allsopp, 1948; Narpozzi, 1953); but they produce tumours rather slowly when administered by injection (Boyland and Horning, 1949; Griffin, Brandt and Tatum, 1950, 1951).



XXIV



XXV

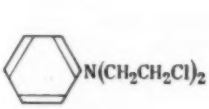


XXVI

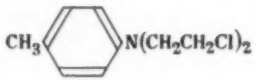
Boyland and Horning used two groups of twenty stock mice. One group was given an aqueous solution of methyl di(2-choroethyl)-amine hydrochloride (XXIV) by subcutaneous injection and received 1·0 mg./kg. at weekly intervals for 50 weeks. The second group received a similar injection of tri(2-chloroethyl)amine hydrochloride (XXV); but the injections were stopped after 10 weeks as only 4 mice remained alive. Of the 14 mice from the two groups which survived more than 250 days, 10 had tumours. These included lung tumours (8), lymphosarcomas (2), a uterine fibromyoma and a spindle-celled sarcoma at the site of injection.

In strain A mice, Heston (1949) obtained tumours after 16 weeks in 100 per cent of the animals receiving a total dose of 0·1 mg. of methyl di(2-chloroethyl) amine hydrochloride. Sulphur mustard (XXVI) has also been shown to give lung tumours in a high percentage of the mice receiving the material by subcutaneous injection (Heston, 1950, 1953), and it is also carcinogenic in the rat (Haddow, 1953).

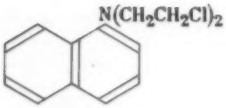
Few aromatic mustards seem to have been tested, but Haddow (1953) has reported that pronounced carcinogenic activity (mainly after subcutaneous injection, but also following feeding) has been shown by several compounds (XXVII–XXXI) of this type.



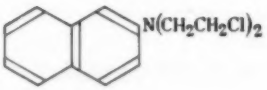
XXVII



XXVIII



XXIX



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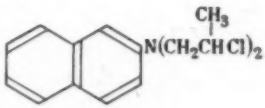
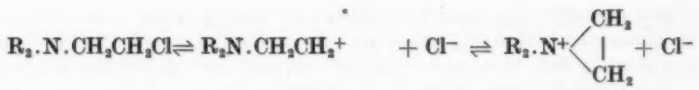


XXXI

The mustards are rather reactive substances, especially in polar solvents. They react with water, with anions and with bases, and in general they can be described as powerful alkylating agents for functional groups such as –OH, $-NH_2$, etc. As such functional groups occur in nucleic acids, in enzymes, and in other important constituents of the cell, the biological activity of the mustards may be intimately related with their chemical reactivity towards such centres. In this connection it may be noted that Ross (1949, 1953) found that there is a good correlation between the rates of hydrolysis of a series of di(2-chloroethyl)amines, $R.N(CH_2CH_2Cl)_2$, and their activity as tumour inhibitors, the most reactive substances being the most active tumour-inhibitors.

The inductive effect of the hetero-atom would be expected to facilitate the removal of the halogen atom to give the carbonium ion (XXXIII), and considerable evidence has accumulated to indicate that this ion is, in fact, the reacting species (Ross, 1953; Alexander, 1954). With aliphatic (but not with aromatic) derivatives, this carbonium ion may be stabilised by passing into the cyclic ethyleneimonium ion (XXXIV).

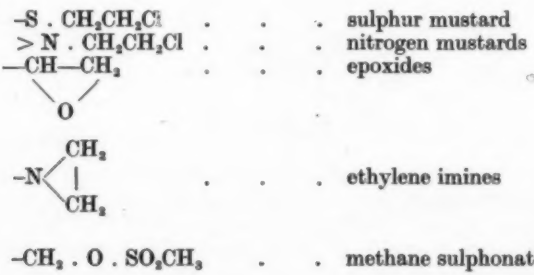


XXXII XXXIII XXXIV

Proof that ionisation does occur was provided by Seligman, Rutenberg and Friedman (1949) who administered diethyl (2-iodoethyl)amine-I^{131}. The resulting radioactivity pattern was found to be almost identical with that shown following administration of radioactive sodium iodide.

This picture of the nitrogen and sulphur mustards as biological alkylating agents suggested that other alkylating agents might also possess similar biological actions. An hypothesis (Goldacre, Loveless and Ross, 1949) that the action might be associated with a cross-linkage reaction directed attention to known cross-linking agents for textile fibres ; but subsequent work has shown that some mono-functional alkylating agents have carcinogenic activity.

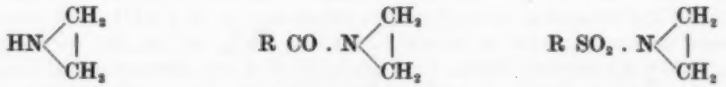
In these groups of alkylating agents the following structural features appear to be the significant ones :


–S . CH2CH2Cl . . . sulphur mustard
> N . CH2CH2Cl . . . nitrogen mustards
—CH—CH2 . . . epoxides
O
–N
CH2
CH2
. . . ethylene imines
–CH2 . O . SO2CH3 . . methane sulphonates.

Ethyleneimine derivatives

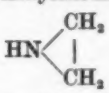
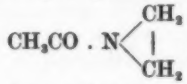
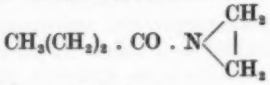
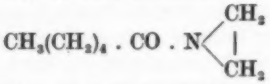
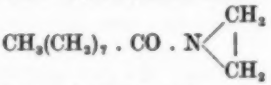
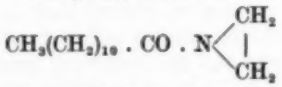
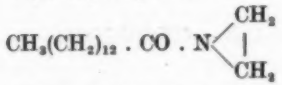
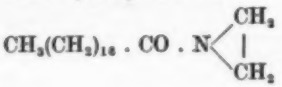
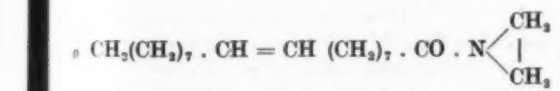
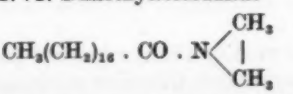
Several N-substituted ethyleneimine derivatives have been tested for carcinogenic activity in both mice and rats (Walpole, Roberts, Rose, Hendry and Homer, 1954), and the results are summarised in Table III.

The compounds were generally tested by subcutaneous injection of their solutions in arachis oil, but the doses and frequencies of injection were not standardised, and it is therefore difficult to assign accurate relative potencies. It seems however that ethyleneimine (XXXV) itself is carcinogenic and that a number of acylethyleneimines (XXXVI) are likewise active. The most potent compounds produced tumours in a high percentage of the rats tested after a latent period of about five months.



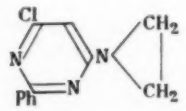
XXXV XXXVI XXXVII

TABLE III.—*Carcinogenic Activity of Ethyleneimine Derivatives.**

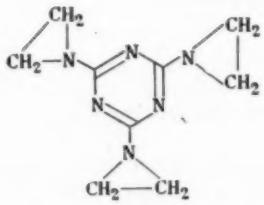
Compound.	Carcinogenic activity.† Mice.	Rats.
Ethyleneimine 	..	++
Acetylethyleneimine 	+	++
Butyrylethyleneimine 	±	++
Caproylethyleneimine 	+	++
Nonanoylethyleneimine 	+	++
Lauroylethyleneimine 	..	±
Myristoylethyleneimine 	++	++
Stearoylethyleneimine 	..	++
	..	+++
N : N-Dimethylstearamide 	..	+

* Walpole, Roberts, Rose, Hendry and Homer (1954).
† Dissolved in arachis oil and injected subcutaneously.

Some attempt was also made to test the analogous ethyleneiminosulphonyl alkanes (XXXVII), but the three compounds tested (R = C_7H_{15}, C_5H_{11} and C_3H_7) all proved to be inactive.



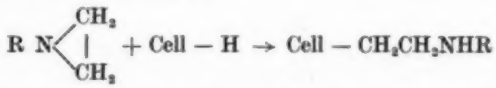
XXXVIII



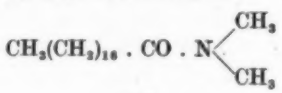
XXXIX

A few ethyleneimine derivatives of a different type hae also been tested. For example, the heterocyclic compound (XXXVIII) was found to be a very potent carcinogen when tested by injection into rats (Walpole, Roberts, Rose, Hendry and Homer, 1954). Triethylenemelamine (*tris*ethyleneimino-*s*-triazine, XXXIX) has been found to induce multiple pulmonary tumours in Strain A mice (Shimkin, 1954), but it seems to be inactive in stock mice (Hendry, Homer, Rose and Walpole, 1951). On the other hand it gives sarcomas at the site of injection in rats and, indeed, is very potent in this respect (Walpole, Roberts, Rose, Hendry and Homer, 1954). Moreover, Roe and Salaman (1955) have found that it has an initiating action on the skin of mice, tumours being produced by subsequent treatment with a promoting agent (croton oil).

It has been suggested (Walpole, Roberts, Rose, Hendry and Homer, 1954) that ethyleneimine may be regarded as the ultimate carcinogen, the function of the acyl group being merely to modulate chemical reactivity and to provide an electrically neutral derivative capable of diffusing readily into the cell. The compounds may be regarded as alkylating agents and the following general scheme may serve as a basis for further work :



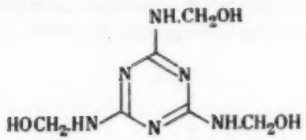
On the other hand, the same authors have found that the three-membered ring system is not essential for carcinogenic activity as N, N-dimethylstearamide (XL) has some carcinogenic activity. However, it is less active than the corresponding cyclic compound, stearoylethyleneimine.



XL

Trimethylolmelamine

Polymethylolamides have been used to modify the mechanical properties of textile fibres, and a number of such substances were therefore submitted for test as tumour-inhibiting agents.

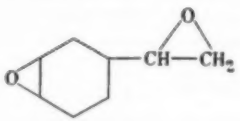


XLI

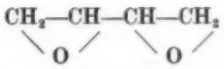
Trimethylolmelamine (XLI) has been tested as a carcinogen in mice and rats (Hendry, Rose and Walpole, 1951). Subcutaneous injection into stock mice resulted, after nine months, in one lymphoid leukaemia, no other mouse showing any sign of malignant disease. Of the 30 rats injected, one developed a highly malignant adenocarcinoma, one a spindle-celled sarcoma at the site of injection, and one developed a lymphoid leukaemia. The authors point out that these tumours may have been spontaneous and do not regard the above results as unequivocal evidence for carcinogenic activity.

Bis-Epoxides

Following the cross-linking hypothesis of Goldacre, Loveless and Ross (1949) bis-epoxides were found to possess the tumour-inhibiting activity and to produce the chromosome abnormalities characteristic of the mustards. Only two compounds of this type seem to have been tested for carcinogenic activity.



XLII



XLIII

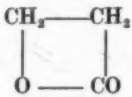
Vinyl *cyclo*hexane dioxide (XLII) has been reported to be a carcinogen for both mice and rats (Hendry, Homer, Rose and Walpole, 1951). This substance was administered to rats by intraperitoneal injection of its solution in arachis oil and a mixed cell sarcoma was obtained in one animal at seven months. When tested in mice by application to the skin, this dioxide seemed to be highly carcinogenic. Of the 20 mice receiving the compound, nine developed malignant tumours and two developed papillomata which regressed when the treatment was stopped. However, Walpole, Roberts, Rose, Hendry and Homer (1954) have repeated the experiment with a more highly purified sample of the diepoxide and have failed to obtain tumours. In the circumstances the carcinogenicity of vinyl *cyclo*hexane dioxide must remain in doubt.

Butadiene dioxide (XLIII) gave no tumours in mice (Hendry, Homer, Rose and Walpole, 1951). A few tumours were observed in rats following intraperitoneal injection of its solution in arachis oil. Here again, however, further experiments are necessary to confirm the carcinogenic activity of this substance.

Propiolactone

The simple aliphatic compound propiolactone (XLIV) was found to be mutagenic (Smith and Srb, 1951) and was therefore tested as a carcinogen. It gave a high

yield of sarcomata following subcutaneous injection in rats and must be rated as a moderately potent carcinogen (Walpole, Roberts, Rose, Hendry and Homer, 1954).

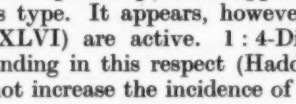


XLIV

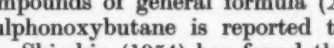
Roe and Salaman (1955) found that it is not carcinogenic to the skin of the mouse; but they were able to show that it does have an initiating action. That is, treatment with propiolactone, followed by croton oil, did produce tumours.

Methane Sulphonates

No complete report has appeared on the carcinogenic activity of compounds of this type. It appears, however, that compounds of general formula (XLV) and (XLVI) are active. 1 : 4-Dimethanesulphonoxybutane is reported to be outstanding in this respect (Haddow, 1953). Shimkin (1954) has found that it does not increase the incidence of pulmonary tumours in strain A mice.



XLV



XLVI

OTHER ORGANIC CARCINOGENS

Senecio *Alkaloids*

Plants of the *Senecio* species are widely used by South African negroes for medicinal purposes, and it seemed probable that this practice might have some bearing on the high incidence of primary liver cancer among these people. In order to test this hypothesis, Cook, Duffy and Schoental (1950) administered the crude alkaloids from *Senecio jacobaea* L. (the common ragwort) in the drinking water, to albino rats. Liver tumours were observed in 3 rats which survived more than eight months of the treatment.

Senecio jacobaea is not itself a native of South Africa, but the alkaloidal constituents are similar to those found in the local species which are commonly used by the natives. More recently, the pure alkaloids retrorsine and isatidine, which are known to occur in South African *Senecio* plants, have also been shown to produce liver tumours (Schoental, Head and Peacock, 1954). In these experiments 58 rats survived the treatment with retrorsine, isatidine or the mixed alkaloids of *Senecio jacobaea* for more than 10 months. Of these, 45 showed changes in the liver ranging from hyperplasia to neoplasia. It is of some interest that tumours were produced in animals receiving a fully adequate diet. A deficient diet of the type known to favour tumour formation by the use of azo-dyes did not prove favourable with the *Senecio* alkaloids.

The alkaloid seneciphylline, also isolated from *Senecio jacobaea*, has been shown to produce liver tumours in fowls (British Empire Cancer Campaign, 1954, p. 4).

The chemistry of the senecio alkaloids (the structures of most being known only imperfectly) has been reviewed by Leonard (1950).

Tannic acid

When injected subcutaneously in rats, tannic acid produces cirrhosis, hepatomas and cholangiomas in the liver; but no local carcinogenic action could be demonstrated (Korpássy and Kovacs, 1949; Korpássy and Mosonyi, 1950). The carcinogenic action on the liver was found to be retarded by a high-casein-low-fat diet, and accelereated by a low-casein high-fat diet (Korpássy and Mosonyi, 1953).

Chloroform and carbon tetrachloride

The repeated oral administration of carbon tetrachloride in olive oil to mice of various strains has been shown to produce hepatomas in a very high percentage of the animals treated (Edwards, 1941; Edwards and Dalton, 1942; Edwards, Edwards, Heston and Dalton, 1942; Eschenbrenner and Miller, 1944).

Similarly the repeated oral administration of chloroform also produces hepatomas and cirrhosis of the liver, provided the individual doses are large enough to produce liver necrosis (Eschenbrenner and Miller, 1945).

These findings are of some importance in view of the widespread medical and industrial use of these materials. It is noteworthy that some hepatic injury has been reported in workers employed in the manufacture of carbon tetrachloride insecticide and fire extinguisher bombs (Sassi and Paruccini, 1954).

The mode of action of these agents remains obscure but it seems that the carcinogenic activity is completely unrelated to the narcotic activity.

Urethanes

The intraperitoneal injection of ethyl urethane (ethyl carbamate, XLVII) has been markedly to increase the incidence of lung tumours in mice (Nettleship, Henshaw and Meyer, 1943).

$$H_2N \cdot COOC_2H_5$$

XLVII

The effect has been found in all the strains of mice tested, but some strains were found to be more susceptible than others. It seems that this susceptibility parallels the susceptibility of the strain to spontaneous pulmonary tumours (Larsen, 1946; Orr, 1947; Cowen, 1947). The effect is unrelated to the anaesthetic action of urethane, and a variety of other hypnotics proved ineffective in increasing the incidence of lung tumours. On the other hand a single anaesthetic dose of urethane sometimes proved effective; moreover the induction period may be as short as two months (Henshaw and Meyer, 1944).

Ethyl urethane has also been found to have a carcinogenic action in rats, whether administered orally or by intraperitoneal injection (Jaffé, 1947; Guyer and Claus, 1947). In both cases a high incidence of lung tumours was observed.

Other esters of carbamic acid have also been tested as pulmonary carcinogens, but the ethyl ester seems to be the most potent. The relative potencies of the ethyl, *iso*propyl and *n*-propyl esters, for example, were found to be of the order of 81, 4 and 1 respectively. Trichloroethyl carbamate was found to be slightly carcino-

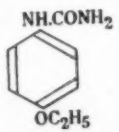
genic (and toxic); but the methyl-monochloroethyl-, *n*-butyl- and *iso*amyl esters were found to be inactive at the dosage levels employed (Larsen, 1947).

The effect of alkyl substitution on the nitrogen atom has also been studied (Larsen, 1948), and in general, changes of this type were found to decrease the activity as pulmonary carcinogens. Ethyl N-methyl carbamate was found to be much less effective than ethyl carbamate itself; and N,N-dialkylated derivatives were slightly less effective than the mono-alkyl compounds. With the exception of the *N*-isopropyl compound, activity decreased as the length of the alkyl group was increased.

In addition to this carcinogenic action towards the lung, it has recently been shown that urethane produces a pre-neoplastic change when applied to the skin of mice. When applied alone to the skin it produces no local tumours; but when such application is followed by treatment with a promoting agent (croton oil) tumours are produced (Salaman and Roe, 1953; Roe and Salaman, 1954). This tumour-initiating action of urethane can be inhibited by the simultaneous administration of glycine and formate, two purine precursors (Roe, 1955). This observation lends some support to the hypothesis that urethane acts by competing with one or more of the precursors in purine synthesis, perhaps leading to an unphysiological purine-like substance (Roe, 1955).

Dulcin

The synthetic sweetening agent Dulcin (*p*-phenetylurea, XLVIII) has some structural similarities to the urethanes, but this is largely superficial.



XLVIII

Fitzhugh and Nelson (1950) have found that this substance produces liver tumours in albino rats fed at dosage levels of 0·1 per cent and above. At 0·5 and 1 per cent, Dulcin retarded the growth rate, increased the mortality rate and produced dark red spleens.

Polymers

In 1941 Turner found that sarcomas are produced in rats following the subcutaneous implantation of Bakelite disks. Of 9 rats which survived longer than 20 months, 4 developed sarcomas at the site of the disk. It has since been found that several other natural and synthetic polymers can produce similar tumours: these include cellophane, polyethylene, polyvinylchloride, polystyrene, polymethyl methacrylate, polytetrafluoroethylene and ε-polycaprolactam (Oppenheimer, Oppenheimer and Stout, 1948, 1952, 1954; Druckrey and Schmähl, 1954; Laskin, Robinson, and Weinmann, 1954).

The pure polymers seem to be as effective as the commerical products, and benzene extracts of such polymers have produced no tumours. Implanted quartz sand usually produced tumours; but glass was found to be ineffective.

The carcinogenic activity of these polymers is somewhat surprising in view of the stability of these materials. Nevertheless polymers do degrade slowly, and it is not unreasonable to suppose that the resulting free radicals will react with important cellular constituents (Oppenheimer, Oppenheimer, Danishefsky, Stout and Eirich, 1955). The polymers do not seem to be very potent carcinogens, producing tumours only after a long latent period. Nevertheless the observations are of considerable importance in view of the use of various plastics in surgery.

SUMMARY

1. Chemical carcinogens other than the polycyclic aromatic hydrocarbons and the azo-compounds, have been reviewed.

2. Inorganic carcinogens include derivatives of arsenic, beryllium, chromium, cobalt, nickel and zinc.

3. Many aromatic amines are cancer-producing. It seems that small changes in chemical structure may affect the distribution of the amine in the body and thus alter the distribution of tumours produced. Hormonal influences also have a profound effect on the distribution.

4. *Ortho*-hydroxylation of aromatic amines is of considerable importance in the carcinogenic process; but not all *o*-hydroxyamines are cancer-producing.

5. Among the cholesterol derivatives, the recent discovery that 6β-hydroperoxy-Δ^4-cholestene-3-one is a carcinogen is of considerable importance.

6. Nitrogen and sulphur mustards, ethyleneimine derivatives, methane sulphonates and propiolactone appear to be carcinogenic; but the present evidence is inconclusive in regard to trimethylolamine and the bis-epoxides.

7. Several *Senecio* alkaloids are active in producing cancer of the liver, and tumours in the same organ are produced by tannic acid, chloroform, carbon tetrachloride, and by the synthetic sweetening agent Dulcin.

8. Certain urethanes (especially ethyl urethane) produce lung tumours in mice. Ethyl urethane has also been shown to have an initiating action on the skin of mice.

9. Several natural and synthetic polymers produce sarcomas following subcutaneous implantation.

REFERENCES

ADLER, O.—(1908) *Arch. exp. Path. Pharmak.*, **58**, 167.
ALEXANDER, P.—(1954) " Advances in Cancer Research ", **2**, 1. New York (Academi Press).
ANISSIMOVA, V.(1939) *Amer. J. Cancer*, **36**, 229.
ARHELGER, S. W. AND KREMEN, A. J.—(1951) *Surgery*, **30**, 977.
ARMSTRONG, E. C. AND BONSER, G. M.—(1944) *J. Path. Bact.*, **56**, 507.—(1947) *Ibid.*, **59**, 19.
BADGER, G. M.—(1948) *Brit. J. Cancer*, **2**, 309.
Idem, COOK, J. W., HEWETT, C. L., KENNAWAY, E. L., KENNAWAY, N. M. AND MARTIN, R. H.—(1942) *Proc. Roy. Soc.*, B, **131**, 170.
Idem AND LEWIS, G. E.—(1952) *Brit. J. Cancer*, **6**, 270.
Idem, LEWIS, G. E., AND REID, R. T. W.—(1954) *Nature*, **173**, 313.
BAETJER, A. M.—(1950) *Arch. industr. Hyg.*, **2**, 487, 505.
BAGG, H. J.—(1936) *Amer. J. Cancer*, **26**, 69.
BAKER, K.—(1950) *Acta Un. int. Cancer*, **1**, 46. (1953) *Cancer Res.*, **2**, 137.

BARNES, J. M.—(1950) *Lancet*, i, 463.
Idem, DENZ, F. A. AND SISSONS, H. A.—(1950) *Brit. J. Cancer*, **4**, 212.
BARRY, G., COOK, J. W., HASLEWOOD, G. A. D., HEWETT, C. L., HIEGER, I. AND KENNAWAY, E. L.—(1935) *Proc. Roy. Soc.*, B. **117**, 318.
BARSOTTI, M. AND VIGLIANI, E. C.—(1952) *Arch. industr. Hyg.*, **5**, 234.
BERENBLUM, I. AND BONSER, G. M.—(1937) *J. industr. Hyg.*, **19**, 86.
BIDSTRUP, P. L.—(1950) *Arch. belges Méd. soc.*, **8**, 500.—(1951) *Brit. J. industr. Med.*, **8**, 302.
BIELSCHOWSKY, F.—(1944*a*) *Brit. J. exp. Path.*, **25**, 1.—(1944*b*) *Ibid.*, **25**, 90.—(1945*a*) *Ibid.*, **26**, 270.—(1945*b*) *Biochem. J.*, **39**, 287.—(1946) *Brit. J. exp. Path.*, 27, 54.—(1947) *Brit. med. Bull.*, **4**, 382.—(1949) *Brit. J. Cancer*, **3**, 547.
Idem AND HALL, W. H.—(1953) *Brit. J. Cancer*, **7**, 358.
BISCHOFF, F. AND RUPP, J. J.—(1946) *Cancer Res.*, **6**, 403.
BONSER, G. M.—(1943) *J. Path. Bact.*, **55**, 1.
Idem, CLAYSON, D. B. AND JULL, J. W.—(1951) *Lancet*, ii, 286.—(1954) *Nature*, **174**, 879.
Iidem AND PYRAH, L. N.—(1952) *Brit. J. Cancer*, **6**, 412.—(1953) *Ibid.*, **7**, 456.
BOURNE, H. G. AND YEE, H. T.—(1950) *Industr. Med.*, **19**, 563.
BOYLAND, E. AND HORNING, E. S.—(1949) *Brit. J. Cancer*, **3**, 118.
BRADSHAW, L. AND CLAYSON, D. B.—(1955) *Nature*, **176**, 974.
British Empire Cancer Campaign (1953) *Ann. Rep.*, **31**, 193. (1954)—*Ibid.*, **32**, 223, 4.
CANTAROW, A., STASNEY, J. AND PASCHKIS, K. E.—(1948) *Cancer Res.*, **8**, 412.
CASE, R. A. M., HOSKER, M. E., MCDONALD, D. B. AND PEARSON, J. T.—(1954) *Brit. J. industr. Med.*, **11**, 75.
CASE, R. A. M. AND PEARSON, J. T.—(1954) *Ibid.*, **11**, 213.
CLAYSON, D. B.—(1950) *Biochem. J.*, **47**, xlvi.—(1953) *Brit. J. Cancer*, **7**, 460.
COOK, J. W.—(1933) *Proc. Roy. Soc.*, B **113**, 277.
Idem, DUFFY, E. AND SCHOENTAL, R.—(1950) *Brit. J. Cancer*, **4**, 405.
Idem AND HASLEWOOD, G. A. D.—(1933) *Chem. & Ind.* (*Rev.*), **52**, 758.—(1934) *J. chem. Soc.*, p. 428.—(1935) *J. Amer. chem. Soc.*, **57**, 1380.
COWEN, P. N.—(1947) *Brit. J. Cancer*, **1**, 401.
DOBRINER, K., HOFMANN, K. AND RHOADS, C. P.—(1941) *Science*, **93**, 600.
DRUCKNEY, H. AND SCHMÄHL, D.—(1954) *Acta Un. int. Cancr.*, **10**, 119; *Naturwissenschaffen*, **41**, 534.
Iidem AND REITER, A.—(1954) *Arzneimittel-Forsch.*, **4**, 365.
DUNNING, W. F., CURTIS, M. R. AND MADSEN, M. E.—(1947) *Cancer Res.*, **7**, 134.
DUPTA, D. N.—(1955) *Nature*, **175**, 257.
DUTRA, F. R. AND LARGENT, E. J.—(1950) *Amer. J. Path.*, **26**, 197.
Iidem AND ROTH, J. L.—(1951) *Arch. Path.*, **51**, 473.
EDWARDS, J. E.—(1941) *J. nat. Cancer Inst.*, **2**, 197.
Idem AND DALTON, A. J.—(1942) *Ibid.*, **3**, 19.
Idem, HESTON, W. E. AND DALTON, A. J.—(1942) *Ibid.*, **3**, 297.
ESCHENBRENNER, A. B. AND MILLER, E.—(1944) *Ibid.*, **4**, 385.—(1945) *Ibid.*, **5**, 251.
FALIN, L. I. AND GROMZEWA, K. E.—(1939) *Amer. J. Cancer*, **36**, 233.
FALK, H. L., GOLDFEIN, S. AND STEINER, P. E.—(1939) *Cancer Res.*, **9**, 438.
FELL, H. B. AND ALLSOPP, C. B.—(1948) *Cancer Res.*, **8**, 177.
FIESER, L. F.—(1954) *Science*, **119**, 719.
Idem, GREENE, T. W., BISCHOFF, F., LOPEZ, G. AND RUPP, J. J.—(1955) *J. Amer. chem. Soc.*, **77**, 3928.
FITZHUGH, O. G. AND NELSON, A. A.—(1948) *Science*, **108**, 626.—(1950) *Feder. Proc.*, **9**, 272.
FOULDS, L.—(1947) *Brit. J. Cancer*, **1**, 172.
GARDNER, L. U. AND HESLINGTON, H. F.—(1946) *Feder. Proc.*, **5**, 221.
GOLDBLATT, M. W.—(1949) *Brit. J. industr. Med.*, **6**, 65.

GOLDACRE, R. J., LOVELESS, A. AND ROSS, W. C. J.—(1949) *Nature*, **163**, 667.
GRAHAM, J. D. P. AND HOOD, J.—(1948) *Brit. J. Pharmacol.*, **3**, 84.
GRIER, R. S., NASH, P. AND FREIMAN, D. G.—(1948) *J. industr. Hyg.*, **30**, 228.
GRIFFIN, A. C., BRANDT, E. L. AND TATUM, E. L.—(1950) *J. Amer. med. Ass.*, **144**. 571.—(1951) *Cancer Res.*, **11**, 253.
GUYER, M. F. AND CLAUS, P. E.—(1947) *Cancer Res.*, **7**, 342.
HADDOW, A.—(1953) 'The Physiopathology of Cancer' (edited Homburger and Fishman) New York (Hoeber).
Idem, HARRIS, R. J. C., KON, G. A. R. AND ROE, E. M. F.—(1948) *Phil. Trans.*, **241**, 147.
HALL, W. H.—(1948) *Brit. J. Cancer*, **2**, 273.
HARRIS, P. N.—(1947) *Cancer Res.*, **7**, 88.
HEATH, J. C.—(1954) *Nature*, **173**, 822.
HENDRY, J. A., HOMER, R. F., ROSE, F. L. AND WALPOLE, A. L.—(1951) *Brit. J. Pharmacol.*, **6**, 357.
HENDRY, J. A., MATTHEWS, J. J., WALPOLE, A. L. AND WILLIAMS, M. H. C.—(1955) *Nature*, **175**, 1131.
HENDRY, J. A., ROSE, F. L. AND WALPOLE, A. L.—(1951) *Brit. J. Pharmacol.*, **6**, 20.
HENSHAW, P. S. AND MEYER, H. L.—(1944) *J. nat. Cancer Inst.*, **4**, 523.
HESTON, W. E.—(1949) *Ibid.*, **10**, 125.—(1950) *Ibid.*, **11**, 415.—(1953) *Proc. Soc. exp. Biol., N.Y.*, **82**, 457.
HIEGER, I.—(1946) *Cancer Res.*, **6**, 657.—(1947) *Nature*, **160**, 270.—(1949) *Brit. J. Cancer*, **3**, 123.
Idem AND ORR, J. W.—(1954) *Brit. J. Cancer*, **8**, 274.
HOAGLAND, M. B., GRIER, R. S. AND HOOD, M. B.—(1950) *Cancer Res.*, **10**, 629.
HUEPER, W. C.—(1938) *Arch. Path.*, **25**, 856.—(1942) "Occupational Tumors and Allied Diseases', Springfield, Illinois (Charles C. Thomas) 896 pp.—(1952) *Tex. Rep. Biol. Med.*, **10**, 167.—(1954) *Arch. Path.*, **58**, 360, 475, 645.
Idem, WILEY, F. H. AND WOLFE, H. D.—(1938) *J. industr. Hyg.*, **20**, 46.
Idem, ZUEFLE, J. H., LINK, A. M. AND JOHNSON, M. G.—(1952) *J. nat. Cancer Inst.*, **13**, 291.
JAFFÉ, W. G.—(1947) *Cancer Res.*, **7**, 107.
KENNAWAY, E. L. AND COOK, J. W.—(1932) *Chem. & Ind. (Rev.)*, **51**, 521.
KENNAWAY, E. L. AND SAMPSON, B.—(1928) *J. Path. Bact.*, **31**, 609.
KIRBY, A. H. M.—(1947) *Brit. J. Cancer*, **1**, 68.—(1948) *Ibid.*, **2**, 294.
Idem AND PEACOCK, P. R.—(1949) *Glasg. med. J.*, **30**, 364.
KORPÁSSY, B. AND KOVÁCS, K.—(1949) *Brit. J. exp. Path.*, **30**, 266.
Idem AND MOSONYI, M.—(1950) *Brit. J. Cancer*, **4**, 411.—(1953) *Kiserl. Orvostud*, **5**, 258.
LARIONOV, L. F.—(1930) *Voprosy Onkologii, Kharkov*, **3**, 114; *Amer. J. Cancer*, **15**, 2832 (1931).
LARSEN, C. D.—(1946) *Cancer Res.*, **6**, 500 Proc.—(1947) *J. nat. Cancer Inst.*, **8**, 99.—(1948) *Ibid.*, **9**, 35.
LASKIN, D. M., ROBINSON, I. B. AND WEINMANN, J. P.—(1954) *Proc. Soc. exp. Biol. N.Y.*, **87**, 329.
LEONARD, N. J.—(1950) 'The Alkaloids', edited Manske and Holmes. New York (Academic Press). Vol. 1, p. 107.
MACHLE, W. AND GREGORIUS, F.—(1948) *Publ. Hlth Rep. Wash.*, **63**, 1114.
MANCUSO, T. F. AND HUEPER, W. C.—(1931) *Industr. Med.*, **20**, 358.
MANSON, L. A. AND YOUNG, L.—(1950) *Biochem. J.*, **47**, 170.
MICHALOWSKY, I.—(1928) *Virchows Arch.*, **267**, 27.
MILLER, E. C., MILLER, J. A., SANDIN, R. B. AND BROWN, R. K.—(1949) *Cancer Res.*, **9**, 504.
MILLER, J. A., SANDIN, R. B., MILLER, E. C. AND RUSCH, H. P.—(1955) *Ibid.*, **15**, 188.
MORRIS, H. P., WEISBURGER, J. H. AND WEISBURGER, E. K.—(1950) *Ibid.*, **10**, 620.

NARAT, J. K.—(1925) *J. Cancer Res.*, **9**, 135.
NARPOZZI, A.—(1953) *Riv. Anat. pat. Oncol.* (*Padova*), **6**, 1155.
NASH, P.—(1950) *Lancet*, i, 519.
NETTLESHIP, A., HENSHAW, P. S. AND MEYER, H. L.—(1943) *J. nat. Cancer Inst.*, **4**, 309.
NEUBAUER, O.—(1947) *Brit. J. Cancer*, **1**, 192.
OPPENHEIMER, B. S., OPPENHEIMER, E. T. AND STOUT, A. P.—(1948) *Proc. Soc. exp. Biol. N.Y.*, **67**, 33.—(1952) *Ibid.*, **79**, 366.—(1954) *Surg. Forum*, **4**, 672.
OPPENHEIMER, B. S., OPPENHEIMER, E. T., DANISHEFSKY, I., STOUT, A. P. AND EIRICH F. R.—(1955) *Cancer Res.*, **15**, 333.
ORR, J. W.—(1947) *Brit. J. Cancer*, **1**, 311.
PEACOCK, P. R.—(1947) *Brit. med. Bull.*, **4**, 364.
PULLMAN, A. AND PULLMAN, B.—(1955) ' Cancérisation par les Substances Chimiques et Structure Moléculaire '. Paris (Masson et Cie).
PURVES, H. D. AND GRIESBACH, W. E.—(1947) *Brit. J. exp. Path.*, **28**, 46.
PUTMAN, F. L.—(1945) *Dermat. Syph.*, **51**, 221.
REHN, L.—(1895) *Arch. klin. Chir.*, **50**, 588.
ROBERTSON, J. W. AND CLEMENT, K. W.—(1948) *Ohio St. med. J.*, **44**, 280.
ROE, F. J. C.—(1955) *Nature*, **175**, 636.
Idem AND SALAMAN, M. H.—(1954) *Brit. J. Cancer*, **8**, 666.—(1955) *Ibid.*, **9**, 177.
ROSS, W. C. J.—(1949) *J. chem. Soc.*, p. 183.—(1953) ' Advances in Cancer Research ', **1**, 397. New York (Academic Press).
RUSSELL, B., GREEN, B. AND WAND, L. G. R.—(1948) *Lancet*, ii, 169.
SALAMAN, M. H. AND ROE, F. J. C.—(1953) *Brit. J. Cancer*, **7**, 472.
SANDIN, R. B., MELBY, R., HAY, A. S., JONES, R. N., MILLER, E. C. AND MILLER, J. A.—(1952) *J. Amer. chem. Soc.*, **74**, 5073.
SASSI, C. AND PARUCCINI, C.—(1954) *Med. d. Lavoro*, **45**, 93 ; *Chem. Abs.*, **48**, 9585.
SCHOENTAL, R., HEAD, M. A. AND PEACOCK, P. R.—(1954) *Brit. J. Cancer*, **8**, 458.
SCOTT, T. S.—(1952) *Brit. J. industr. Med.*, **9**, 127.
SELIGMAN, A. M., RUTENBERG, A. M. AND FRIEDMAN, O. M.—(1949) *J. nat. Cancer Inst.*, **9**, 261.
SHEAR, M. J.—(1938) *J. biol. Chem.*, **123**, *Proc.* cviii.
SHIMKIN, M. B.—(1954) *Cancer*, **7**, 410.
SISSONS, H. A.—(1950) *Acta Un. int. Cancer*, **7**, 171.
SMITH, H. H. AND SRB, A. M.—(1951) *Science*, **114**, 490.
SNEGIREFF, L. S. AND LOMBARD, O. M.—(1951) *Arch. industr. Hyg.*, **4**, 199.
SUNTZEFF, V., BABCOCK, R. S. AND LOEB, L.—(1940) *Amer. J. Cancer*, **39**, 56.
SPIELMAN, M. A. AND MEYER, R. K.—(1939) *J. Amer. chem. Soc.*, **61**, 893.
SPITZ, S., MAGUIGAN, W. H. AND DOBRINER, K.—(1950) *Cancer*, **3**, 789.
THOMPSON, R. H. S. AND WHITTAKER, V. P.—(1947) *Biochem. J.*, **41**, 342.
TURNER, F. C.—(1941) *J. nat. Cancer Inst.*, **2**, 81.
VAN ORDSTRAND, H. S., HUGHES, R., DENARDI, J. M. AND CARMODY, M. G.—(1945) *J. Amer. med. Ass.*, **129**, 1084.
WALPOLE, A. L., ROBERTS, D. C., ROSE, F. L., HENDRY, J. A. AND HOMER, R. F.—(1954) *Brit. J. Pharmacol.*, **9**, 306.
WALPOLE, A. L., WILLIAMS, M. H. C. AND ROBERTS, D. C.—(1952) *Brit. J. industr. Med.*, **9**, 255.—(1954) *Ibid.*, **11**, 105.—(1955) *Brit. J. Cancer*, **9**, 170.
WEISBURGER, E. K.—(1955) *J. Amer. chem. Soc.*, **77**, 1914.
Idem AND WEISBURGER, J. H.—(1954) *J. org. Chem.*, **19**, 964.—(1955) *Ibid.*, **20**, 1396.
WIELAND, H. AND DANE, E.—(1933) *Z. physiol. Chem.*, **219**, 240.
WILEY, F. H.—(1938) *J. biol. Chem.*, **124**, 627.
WILSON, R. H., DEEDS, F. AND COX, A. J.—(1941) *Cancer Res.*, **1**, 595.—(1947*a*) *Ibid.*, **7**, 444.—(1947*b*) *Ibid.*, **7**, 453.

THE CARCINOGENICITY OF β-PROPIOLACTONE FOR MOUSE-SKIN

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Received for publication March 2, 1956.

IN a previous communication from this laboratory (Roe and Salaman, 1955, p. 193) it was reported that applications to the dorsal skin of the mouse of β-propiolactone and croton oil, alternately at 3- to 4-day intervals, gave rise to significantly more papillomata than equivalent treatment with croton oil only. It was concluded that β-propiolactone is, like urethane (Salaman and Roe, 1953; Graffi *et al.* 1953; Berenblum and Haran, 1955), triethylene melamine (Roe and Salaman, 1955), and 1,2-benzanthracene (Graffi *et al.*, 1953; Roe and Salaman, 1955), an initiator of carcinogenesis in mouse-skin.

It was pointed out (Roe and Salaman, 1955, p. 198) that in the group of mice treated with both β-propiolactone and croton oil papillomata appeared as early as the second week of croton oil treatment. This early appearance of papillomata, and the fact that 4 malignant tumours were present in 19 survivors only 25 weeks from the beginning of the experiment*, suggested that β-propiolactone might prove to be carcinogenic for mouse-skin, as it is for the subcutaneous tissues of the rat (Walpole *et al.*, 1954).

In an addendum (Roe and Salaman, 1955, p. 201) an experiment was described in which 10 mice were painted weekly for over 20 weeks with 2·5 per cent β-propiolactone only. No tumours were observed in these mice up to the time of going to press. However, tumours began to appear at a later date, and details of these are given below in the first experimental section.

A second possible explanation of the early appearance of papillomata and malignant tumours in the mice treated with β-propiolactone and croton oil is that the scarring caused by the first two applications of the former (at 10 per cent and 5 per cent, respectively) exerted a promoting effect on tumour production. This possibility is investigated in the latter part of the first experimental section below.

In an attempt to correlate the ability to initiate tumour-formation in mouse skin and certain other properties (Roe and Salaman, 1955, p. 198) it was suggested that there is a positive correlation between tumour-initiating activity for mouse skin and adenoma-inducing activity for mouse lung. However, as reported in the addendum referred to, only 1 adenoma was seen at post-mortem in 18 mice painted with β-propiolactone and croton oil.

A possible explanation of the failure of skin-applications of β-propiolactone to give rise to lung tumours is that it may be totally detoxicated in the skin, with

* Four more malignant tumours arose in this group of mice between the 25th and 30th weeks. Altogether 6 mice bore malignant tumours, 2 of them bearing 2 each. All the 8 malignant tumours were of epithelial origin, and had penetrated the panniculus carnosus; no metastases were seen. The experiment was terminated at the end of the 30th week.

the result that none reaches the lung in an active form. To exclude this possibility an experiment was designed in which β-propiolactone was administered intravenously via the tail vein. The local effect on the skin at the injection site, and the effect on lung tumour formation, of the intravenous injection of β-propiolactone are described in the second experimental section.

MATERIALS AND METHODS

Mice.—Male and female stock albino mice of the "S" strain were used. Details of the care, feeding, and vaccination as a precaution against ectromelia, are given in a previous communication (Roe and Salaman, 1955).

Chemical substances and their administration.—The sources of β-propiolactone, croton oil, and acetone, and the technique of application to the skin, have been fully described elsewhere (Roe and Salaman, 1955).

Before intravenous injection mice were warmed in an incubator at 37° C. for a few minutes. Injection was made into the dorsal vein about 3 cm. from the base of the tail (proximal to the scar made by previous vaccination).

Methods.—The methods used for the recording of skin tumours, for the examination of mice for lung tumours at post-mortem, and for the histological examination of tissues, are fully described in a previous communication. (Roe and Salaman, 1955).

EXPERIMENTAL

I. *The Carcinogenic Effect of β-propiolactone on Mouse Skin*

(a) *The effect of β-propiolactone applied repeatedly at a subulcerative concentration (2·5 per cent)*

Ten mice were painted weekly with 0·3 ml. 2·5 per cent β-propiolactone in acetone for 52 weeks. During this period and for 3 weeks afterwards mice were examined at weekly intervals for tumours of the skin.

One mouse died after 5 weeks, the remaining 9 lived until 47 applications had been given. In these, papillomata began to appear after 27 applications, and altogether 5 mice bore papillomata. During the 40th week one papilloma on each of 2 of these 5 mice underwent a malignant change. Both tumours were removed surgically under anaesthesia, but both recurred. One metastasised to regional glands. Three weeks after the end of treatment (55th week) there were only 4 survivors, and these had to be killed because of their poor general condition.

The two malignant tumours were examined histologically; one was a highly anaplastic carcinoma and the other a moderately well-differentiated tumour. Both had penetrated the panniculus carnosus. Fig. 1 and 2 were prepared from a section of the more differentiated tumour.

Histological examination showed that two other tumours had infiltrated the dermis but had not reached the panniculus carnosus. These were regarded as "probably" malignant (Roe, 1956).

(b) *The effect of early scarring, due to high concentration of β-propiolactone, on the carcinogenic effect of repeated applications of the latter*

Twenty mice were given 5 weekly applications of β-propiolactone at concentrations sufficient to produce and maintain moderate ulceration and scabbing on

a majority of the mice (10, 5, 5, 10, and 5 per cent respectively). Since then, weekly applications of β-propiolactone have been continued at a sub-ulcerative concentration (2·5 per cent). During this latter treatment the ulcers and scabs caused by the earlier treatment disappeared in all mice except one. The remaining mouse was left with a curved linear scar, 2·5 cm. long, which passed diagonally across the back from the left scapula to the middle of the haunches, and remained clearly visible. Seven weeks after the beginning of treatment a papilloma appeared alongside the scar, a second papilloma appeared in a similar position after 12 weeks, and a third 3 weeks later. All three tumours, arranged in a line alongside the scar, were sessile and grew rapidly. By the 21st week all were ulcerated and obviously malignant. The mouse was killed 2 weeks later. Histological examination showed the central tumour to be an anaplastic carcinoma, and the other two to be squamous carcinomata. All had penetrated the panniculus carnosus, but no metastases were seen.

A second mouse, in which no visible scar persisted, developed a papilloma on the back during the 22nd week of the experiment. This tumour enlarged steadily, and by the 31st week appeared malignant to the naked-eye. By this time a second tumour, a papilloma, had appeared. Two weeks later the mouse died, but advanced post-mortem changes were present when its death was discovered, and no sections could be taken of the two tumours.

The experiment is at present in its 40th week, and there are 4 mice still alive. Thirteen mice have so far died tumourless.

It is concluded that β-propiolactone applied repeatedly over a period is carcinogenic for mouse skin. Not many tumours arise, but most of those that do undergo malignant transformation within a few weeks of their first appearance.

A scar caused by the application of β-propiolactone in high concentration was the site of 3 tumours, all of which appeared early and all of which became malignant.

II. *The effects of Intravenous Injection of β-propiolactone*

(a) *Formation of skin tumours at the site of intravenous injection of β-propiolactone*

The purpose of the following experiment was to determine whether the intravenous administration of β-propiolactone to mice would lead to the formation of lung tumours. Although the results in this respect were negative (*vide infra*), the early appearance of tumours of the skin at the site of injection on the tail was thought worthy of description.

Thirty-two mice were injected intravenously by the tail vein with β-propiolactone in sterile Ringer solution as follows :

1 mg./0·1 ml. (3 ♂ mice.)
3 ,, / ,, ,, (3 ♂ ,,)
5 ,, / ,, ,, (10 ♀ ,, and 3 ♂ mice.)
6 ,, /0.2 ,, (3 ♂ ,,)
10 ,, / ,, ,, (10 ♀ ,,)

Three of the mice given 10 mg. β-propiolactone became miserable and hunched during the 24 hours after injection. Two of these recovered during the next few days, the third, and another mouse which at first appeared well, died. None of the

other mice showed any signs of general intoxication. The tails of almost all the mice showed moderate or severe inflammatory changes in the neighbourhood of the site of injection. In 13 mice this inflammation proceeded to gangrene and the partial or complete loss of the tail. The records of a few of the mice which lost their tails indicated that some of the injected material had been accidently introduced outside the vein; in the others, where no appreciable extravenous injection occurred, it is thought probable that some of the material leaked out of the vein after injection. Obvious scars persisted at the site of injection on 15 of the 17 survivors which retained their tails. Papillomata arose at the edges of these scars in 3 mice; the first, 8 weeks after the injection of 5 mg. β-propiolactone; the second, 9 weeks after the injection of 10 mg.; and the third, 15 weeks after the injection of 1 mg. (Fig. 3). The first of these three mice developed two further papillomata close to the scar; these persisted for several weeks, but eventually sloughed off when the area of skin which bore them became ulcerated. Forty weeks after injection this mouse was killed; sections taken from the ulcerated area showed chronic inflammatory changes only. The mouse which developed a papilloma at the injection site after 9 weeks developed a second similar tumour on the skin of the base of the tail overlying the vein into which the injection was made, but some distance from the point of insertion of the needle (Fig. 4). During the 48th week the first tumour in this mouse began to expand rapidly, and two weeks later the tail was amputated. Histologically the tumour, which had invaded the vertebrae, consisted of spindle cells of uncertain origin.

(b) *Failure to induce the formation of lung tumours by the intravenous injection of β-propiolactone*

As described in the previous section a total of 32 mice were given a single intravenous injection, via the tail vein, of β-propiolactone in sterile Ringer solution, in doses ranging from 1 to 10 mg.

Six months after injection 18 of the 26 survivors were killed and examined post-mortem for the presence of lung adenomata. (The remaining 8 mice are still under observation.) One mouse bore 5 lung tumours, 4 mice bore one each, and 13 mice bore none. This incidence of pulmonary adenomata is within normal limits for untreated mice of the same strain and sex.

It is concluded that single intravenous injections of β-propiolactone, in doses ranging from 1 to 10 mg., do not increase the incidence of pulmonary adenomata in "S" strain mice. On the other hand, contamination of the skin and of the subcutaneous tissues during the intravenous injection of β-propiolactone into the

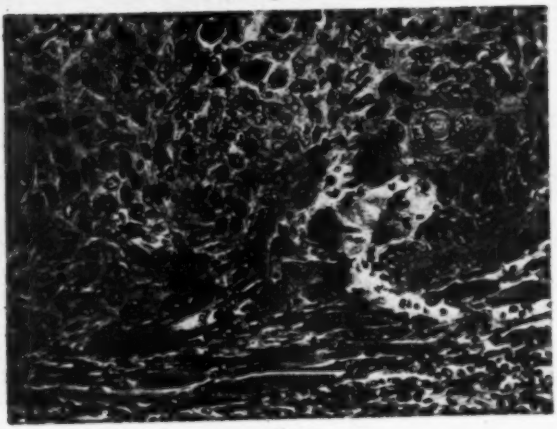
EXPLANATION OF PLATES.

FIG. 1 and 2.—Squamous-cell carcinoma which arose on the back of a mouse after 40 weekly applications of 2·5 per cent β-propiolactone in acetone. Fig. 1 shows tumour tissue reaching down to the level of the panniculus carnosus. × 12. Fig. 2 shows infiltration by tumour cells of this muscle layer. × 270. [Staining: Haematoxylin and Eosin-Biebrich-scarlet (Salaman and Gwynn, 1951).]

FIG. 3.—Papilloma which arose 15 weeks after the intravenous injection, by the tail vein, of 1·0 mg. β-propiolactone in 0·1 ml. sterile Ringer solution.

FIG. 4.—Papillomata on the tail of a mouse following intravenous injection of 10 mg. β-propiolactone in 0·2 ml. sterile Ringer solution. The larger, more distal, tumour is situated at the site of injection; the smaller tumour has arisen from the skin overlying the injected vein.

1



2

3

4

tail vein, or scarring following such contamination, favours the early development of papillomata at or near the site of injection.

DISCUSSION

The results of the experiments described above are clear-cut : (i) β-propiolactone is carcinogenic for mouse-skin, (ii) scarring of the skin caused by high concentrations of β-propiolactone predisposes to the early appearance of tumours, and (iii) the intravenous administration of β-propiolactone in doses up to 10 mg. does not increase the incidence of pulmonary tumours in mice.

Walpole *et al.* (1954) described the occurrence of local sarcomata in rats following repeated subcutaneous injections of β-propiolactone. The finding that the same substance is carcinogenic for mouse-skin is therefore not entirely unexpected. Nevertheless the fact that this simple substance (molecular weight = 72) is carcinogenic for two different tissues in two distinct species is of considerable interest.

The fact that tumours arose in relation to scars caused by high concentrations of β-propiolactone may be explained in one of two ways : the five weeks of treatment at high concentrations of β-propiolactone may have been disproportionately more effective than prolonged treatment at a lower concentration, or the scarring which resulted from the high concentration may have promoted the early appearance of tumours. The close proximity of the tumours to the scars, both on the back and at the site of injection on the tail, suggest that scarring had some tumour-promoting effect. Such an effect has been the subject of many researches in the past (Pullinger, 1943, 1945*a*, 1945*b* ; Linell, 1947). Further studies of the effect of scarring on the carcinogenic action of β-propiolactone are planned.

In the experiments described above the first papilloma in a mouse painted with β-propiolactone alone appeared 7 weeks after 120 mg. had been applied. It is possible that this dose was in excess of that required to produce tumours. Nevertheless it is probable that the carcinogenicity of β-propiolactone is of a low order compared with that of 9,10-dimethyl-1, 2-benzanthracene (DMBA) ; for instance, a single application (0·3 mg.) of which has been shown to give rise to benign and malignant skin tumours (Roe, 1956). Compared with its apparently weak carcinogenic action the tumour-initiating action of β-propiolactone is strong : a single application of 7·5 mg. followed by 18 weekly applications of croton oil (0·3 ml., 0·5 per cent) gave rise to 22 papillomata* on 9 surviving mice (Roe and Salaman, 1955, p. 201). Its weakness as a carcinogen for mouse skin may be due to a deficiency in promoting power. This suggestion is supported by an experiment of our colleague Mr. R. H. Gwynn (1954, unpublished data). He applied 0·72 per cent β-propiolactone weekly, following a single initiating dose of DMBA. No tumours appeared during 20 weeks of treatment, and he concluded that β-propiolactone at this concentration does not promote tumour development. On the other hand the fact that β-propiolactone may give rise to tumours of mouse-skin by itself indicates that it is not entirely devoid of promoting activity, as appears to be the case with urethane (Salaman and Roe, 1953).

It has been suggested (Roe and Salaman, 1955) that there is a positive correlation between tumour-initiating activity for mouse skin and adenoma-inducing

* After 41 weeks, one of these papillomata showed signs of malignancy, and on microscopic examination showed penetration of the panniculus carnosus.

activity for mouse lung. The classical report of Andervont and Shimkin (1940) indicated that the intravenous route is suitable for testing substances for carcinogenic action on mouse lung, and therefore the failure to produce lung tumours in mice by the intravenous injection of β-propiolactone does not support the suggested correlation. It may be argued that failure was due to insufficient β-propiolactone reaching the lung. However this seems unlikely, since the 10 mg. intravenous doses were near the upper limit of toleration from the point of view of general toxicity.

SUMMARY

1. Weekly applications of 2·5 per cent β-propiolactone to the backs of mice gave rise to papillomata after 27 weeks, and to epitheliomata after 40 weeks.

2. When higher concentrations (5 to 10 per cent) of β-propiolactone were given for the first 5 weeks, followed by weekly applications of 2·5 per cent, ulceration and scarring occurred. In one mouse which bore a clearly visible linear scar on the back, papillomata began to appear alongside the scar after only 7 weeks from the beginning of treatment. Three such tumours had appeared by the 15th week, and by the 21st week all three were malignant.

3. Single intravenous injections of β-propiolactone, in doses ranging from 1 mg. to 10 mg. failed to increase the incidence of pulmonary adenomata, but gave rise to severe inflammation at the site of injection, which was followed by gangrene in some mice and scar-formation in others. Papillomata arose next to the scars in 3 of the latter.

4. These findings are discussed.

We thank Dr. M. H. Salaman for his advice. The expenses of this research were partly defrayed out of a block grant from the British Empire Cancer Campaign.

REFERENCES

Andervont, H. B. and Shimkin, M. B.—(1940) *J. nat. Cancer Inst.*, **1**, 225.
Berenblum, I. and Haran, N.—(1955) *Brit. J. Cancer*, **9**, 453.
Graffi, A., Vlamynch, E., Hoffman, F. and Schultz, I.—(1953) *Arch. Geschwulstforsch.*, **5**, 110.
Linell, F.—(1947) *Acta path. microbiol. scand.*, Suppl., **71**, 1.
Pullinger, B. D.—(1943) *J. Path. Bact.*, **55**, 301.—(1945*a*) *Ibid.*, **57**, 467.—(1945*b*) *Ibid.*, **57**, 477.
Roe, F. J. C.—(1956) *Brit. J. Cancer*, **10**, 61.
Idem and Salaman, M. H.—(1955) *Ibid.*, **9**, 177.
Salaman, M. H. and Gwynn, R. H.—(1951) *Ibid.*, **5**, 252.
Idem and Roe, F. J. C.—(1953) *Ibid.*, **7**, 472.
Walpole, A. L., Roberts, D. C., Rose, F. L., Hendry, J. A. and Homer, R. F. (1954) *Brit. J. Pharmacol.*, **9**, 306.

FURTHER TESTS FOR TUMOUR-INITIATING ACTIVITY: N,N-DI-(2-CHLOROETHYL)-*P*-AMINOPHENYLBUTYRIC ACID (CB1348) AS AN INITIATOR OF SKIN TUMOUR FORMATION IN THE MOUSE

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Received for publication April 17, 1956.

In previous papers (Salaman and Roe, 1953; Roe and Salaman, 1954, 1955), it has been shown that there exist substances which, though not carcinogenic for mouse skin, produce a change in this tissue such that a subsequent short course of croton oil applications, insufficient to produce more than a few tumours in previously untreated skin (Roe, 1956*b*), elicits tumours in considerable numbers. This change was considered to be a form of incomplete carcinogenesis. In accordance with accepted terminologies (Friedewald and Rous, 1944; Furth, 1953) such substances were called " tumour-initiators ".

Urethane (ethyl carbamate), 1, 2-benzanthracene, triethylene melamine (TEM), and β-propiolactone have been shown to be tumour-initators for the skin of the mouse. Two derivatives of urethane (ethyl N-phenylcarbamate and ethyl N-methylcarbamate) showed similar activity, but the results required confirmation (Graffi *et al.*, 1953; Salaman and Roe, 1953; Roe and Salaman, 1955).

In the cases of urethane, and TEM, application to the skin in near-lethal doses without croton oil gave rise to no tumours (nor to any recognisable histological change in the skin); and it was concluded that these substances are non-carcinogenic for mouse skin. On the other hand both gave rise to tumours of mouse lung, and TEM has been shown to give rise to sarcomata when injected subcutaneously in the rat (Walpole *et al.*, 1954). 1,2-Benzanthracene is probably very weakly carcinogenic for mouse skin and other tissues (see Steiner and Falk, 1951, for a review of the evidence); but in contrast to its weak carcinogenic action, its tumour-initiating action is strong. β-Propiolactone, which had not been tested for carcinogenicity in mouse-skin at the time its tumour-initiating effect was described (Roe and Salaman, 1955), has subsequently been found to be weakly carcinogenic for this tissue (Roe and Glendenning, 1956). It had previously been found to give rise to sarcomata following subcutaneous injection in the rat (Walpole *et al.*, 1954). As in the case of 1,2-benzanthracene, the tumour-initiating action of β-propiolactone in mouse skin is considerably stronger than its carcinogenic action.

It was suggested (Roe and Salaman, 1955) that there might be a relation between tumour-initiating activity for mouse skin and tumour-inducing activity for mouse lung.Ureth ane, TEM, and ethyl N-methylcarbamate possess both activities; 1,2-benzanthracene definitely possesses the first and possibly the second; many other substances were shewn to be without either action (including Myleran and colchicine). At the time this suggestion was made it was not known

whether β-propiolactone gave rise to tumours in mouse lung. More recently it has been found that intravenous injections of this substance (1 to 10 mg.) failed to increase the incidence of lung tumours in mice (Roe and Glendenning, 1956). It seems therefore that there is no simple correlation between these two activities. There is at present no clear indication of the chemical basis of tumour-initiating action, and no evidence for a definite relation between tumour-initiation and any other biological activity, except perhaps carcinogenicity for other tissues or species (Roe and Salaman, 1955).

The present paper describes the results of tests of a further 20 substances for tumour-initiating action, and a third test of ethyl N-phenylcarbamate (phenyl-urethane), which gave doubtful and negative results respectively on two previous occasions (Roe and Salaman, 1955, pp. 191 and 201). Reasons for the choice of the 20 substances are briefly outlined below.

Selection of substances

A list of substances screened is given in Table I. It was found convenient to divide them into four classes : *polycyclic hydrocarbons*, *aromatic nitrogen mustards*, *purine analogues*, and *miscellaneous substances*.

In the first class four substances were chosen for test, either because they were thought to be on the borderline between carcinogenicity and non-carcinogenicity, or because they were the parent substances of potent carcinogenic agents. As pointed out above the tumour-initiating action of 1,2-benzanthracene is far stronger than its carcinogenic action, and it was thought possible that the same might be true for other polycyclic hydrocarbons.

Anthracene has been tested for carcinogenicity by many workers, but never with a positive result. Kennaway (1924*a*), who pointed out that workmen handling purified anthracene do not suffer from skin lesions, failed to produce skin tumours in mice with this substance (see also Kennaway, 1924*b* ; Kennaway and Hieger, 1930). Barry *et al.* (1935), and later Bachmann *et al.* (1937), painted mice twice weekly with anthracene in benzene for 88 and 104 weeks respectively, and failed to produce tumours. Boyland and Burrows (1935) injected anthracene in colloidal solution subcutaneously into rats, but observed no tumours. Steiner (1955) injected mice subcutaneously with anthracene in tricaprylin, with a similar result. Demerec (1948) stated that anthracene was probably mutagenic for *Drosophila*, but subsequently withdrew this statement (Demerec, 1949, quoted by Auerbach, 1949).

Deoxycholic acid.—Occasional epitheliomata have been seen in mice painted with deoxycholic acid repeatedly over long periods (Bachmann *et al.*, 1937 ; Badger *et al.*, 1940 ; Cook, Kennaway and Kennaway, 1940), and subcutaneous injection into mice has given rise to sarcomata after a prolonged period (Badger *et al.*, 1940). Klein (1952) found that deoxycholic acid applied to the skin gave rise to no tumours either when used alone or in conjunction with croton oil. Deoxycholic acid has been found to be mutagenic for bacteria (Witkin, 1947 ; Jensen *et al.*, 1951), and for *Drosophila* by Demerec (1948), but not by Jensen *et al.*, (1951).

Phenanthrene has been tested for carcinogenicity both on mouse-skin (Kennaway 1924*a*, 1924*c* ; Kennaway and Hieger, 1930) and also by subcutaneous injection into mice (Steiner, 1955). No tumours have been recorded in any of

TABLE I.—*The Screening of Substances for Tumour-initiating Activity*

Group.	Number of mice.	Treatment with the test substance. Substance.	Total dose.	Method of application.§	Treatment with croton oil.† Reference to control group.	Interval between 1st application of test substance and 1st application of croton oil.	Tumour‡ incidence at the end of croton oil treatment. Survivors.	Tumour-bearing mice.	Total tumours
Class I.—Polycyclic hydrocarbons									
1	20	Anthracene	30·0 mg.	20 applications, 0·5% (2, with an interval of 30 minutes, thrice weekly)	As in Group B	25 days	17	3	4
2	20	Deoxycholic acid	67·5 mg.	10 thrice weekly applications, 2·25% in methanol	„ „ „ B	„ „	20	7	14
3	20	Phenanthrene	0·54 g.	10 thrice weekly applications, 18·0%	„ „ „ B	„ „	20	5	12
4	20	Pyrene	0·25 g.	10 thrice weekly applications 8·3%	„ „ „ B	„ „	20	6	9
Class II.—Aromatic nitrogen mustards									
5	25	NN-di-(2-chloroethyl)-p-aminophenylbutyric acid (CB1348)	2·7 mg.	10 weekly applications, 8 at 0·1%, and 2 at 0·05%, in methanol	As in Group C	30 days*	19	11	30
6	25	*p*-di-(2-chloroethyl)-amino-d-phenylalanine	3·0 mg.	10 weekly applications, 0·1% in methanol	„ „ „ C	„ „ *	22	4	8
7	25	*p*-di-(2-chloroethyl)-amino-l-phenylalanine	1·44 mg.	10 weekly applications, 0·01% to 0·1% in methanol	„ „ „ C	„ „ *	19	2	7

TABLE I—*continued*

Class III.—Purine analogues											
8	25	8-Azaguanine	10 mg.	10 thrice weekly applications, 0·33% in 50% aqueous carbowax 300	As in Group C	23 days	23	2	4
9	25	2,6-Diaminopurine	10 mg.	10 thrice weekly applications, 0·33% in 50% aqueous carbowax 300	„ „ „ C	„ „	23	1	1
10	25	2,6-Diamino-8-phenylpurine	15 mg.	10 thrice weekly applications, 0·5% in 50% aqueous carbowax 300	„ „ „ C	„ „	23	2	2
11	25	6-Mercaptopurine	10 mg.	10 thrice weekly applications, 0·33% in 50% aqueous carbowax 300	„ „ „ C	„ „	18	0	0
Class IV.—Miscellaneous substances									
12	20	Acetylsalicylic acid (aspirin)	0·54 g.	10 thrice weekly applications, 18%	As in Group B	25 days	19	3	5
13	15	Acrolein	12·6 mg.	10 weekly applications, 0·5%	„ „ „ B	„ „ *	15	2	3
14	25	Diethyl carbonate	2·9 g.	10 thrice weekly applications, pure substance	„ „ „ C	23 „	23	2	2
15	20	8-Hydroxyquinoline	9·0 mg.	6 weekly applications, 0·5%	„ „ „ A	25 „ *	14	1	2
16	25	Monosodium maleic hydrazide	0·3 g.	10 thrice weekly applications, 10%, in 50% aqueous carbowax 300	„ „ „ C	23 „	21	2	2
17	20	Oxalacetic acid	160 mg.	10 thrice weekly applications, 5·3%	„ „ „ B	22 „	16	2	3
18	20	Phenylurethane (ethyl N-phenylcarbamate)	1·8 g.	30 applications, 20% (2, with an interval of 30 minutes, each week for 15 weeks)	„ „ „ A	20 weeks	17	8	22
19	14	Potassium arsenite	30 mg.	10 weekly applications, 1% in methanol	„ „ „ B	25 days*	14	3	3
20	20	Saccharin	0·24 g.	10 thrice weekly applications, 8%	„ „ „ B	„ „	20	7	14
21	20	Vanillin	0·6 g.	10 thrice weekly applications, 20%	„ „ „ B	„ „	18	4	7

TABLE I—*continued*

TABLE I—*continued*

Group.	Number of mice.	Treatment		Tumour‡ incidence at the end of croton oil treatment.		
				Survivors.	Tumour-bearing mice.	Total tumours.
Control groups						
A	20	Batch I croton oil only	18 weekly applications, 0·5%	17	1	3
B	20	Batch II croton oil only	1 application of 0·17%; 2 of 0·085%, and a further 15 of 0·17%, at weekly intervals throughout	19	4	4
C	20	Batch II croton oil only	18 weekly applications, 0·17%	17	0	0

§ Each application was 0·3 ml. of solution in acetone (unless otherwise stated) at the concentration shown (w/v in the case of solids, and v/v in the case of liquids).

† Croton oil treatment consisted of 18 weekly applications of 0·3 ml. solution in acetone. Appropriate controls for the test groups are indicated in the Table (Groups A, B, and C).

‡ All the tumours recorded below were benign papillomata.

* The earlier weekly applications of croton oil alternated at 3- to 4- day intervals with those of the test substance.

these tests. Graffi *et al.* (1953) applied phenanthrene and croton oil alternately to the backs of mice for a year; the yield of tumours was not significantly higher than in croton oil controls. Demerec (1948) found phenanthrene to be non-mutagenic for *Drosophila*.

Pyrene.—Occasional papillomata have been seen on mouse skin following repeated applications of pyrene by Barry *et al.* (1935), but not by Badger *et al.* (1940). Graffi *et al.* (1953) tested pyrene in the same way as phenanthrene (see previous paragraph); the result was again negative. Demerec (1948) found it to be non-mutagenic for *Drosophila*.

The second class consisted of three *aromatic nitrogen mustards*. The first of these, *N,N-di-(2-chloroethyl)-p-aminophenylbutyric acid* (*CB*1348), was synthesized by Everett, Roberts, and Ross (1953). It was found to have a more powerful tumour-inhibitory effect on the Walker rat carcinoma 256 than several hundred other biological alkylating agents, including a series of closely related mustards. (Ross *et al.*, 1952). Galton *et al.* (1955) reported striking remissions in patients suffering from Hodgkin's disease, lymphocytic lymphoma, chronic lymphocytic leukaemia, and follicular lymphoma, as a result of treatment with CB1348. Haddow (personal communication) injected a group of male rats subcutaneously weekly for 12 weeks with CB1348 in arachis oil. A sarcoma arose at the injection site in one out of 12 rats one year after the last injection. A sarcoma at the injection site was also seen in one of a group of 12 mice similarly treated. Since it is not unknown for sarcomata to arise following injection of arachis oil only (Walpole *et al.*, 1954) the carcinogenicity of CB1348 remains in doubt. CB1348 was found by Fahmy and Fahmy (1952) to be mutagenic for *Drosophila*, and by Gopal-Ayengar (1952) to produce chromosome abnormalities.

The other two substances of this class are the two isomers *p-di-(2-chloroethyl)-amino-L-*, and *D-phenylalanine*. The synthesis of these substances was described by Bergel and Stock (1954). The same authors (1953) reported a remarkable difference in the tumour-inhibitory effects of the two isomers on the Walker rat carcinoma 256, the L-form showing strong inhibition of growth and the D-form only slight activity. Bergel *et al.*, (1954) state that their colleagues, Dr. Elson and Professor Koller, have found similar differences in the effects of the two isomers on the blood picture of the normal rat, and in the production of chromosome abnormalities.

The isomers have not been tested separately for carcinogenicity, but in an experiment (Haddow: personal communication) in which the racemic mixture was injected subcutaneously in arachis oil weekly for 12 weeks into 10 female rats, a sarcoma arose at the injection site in one rat 18 months after the final injection. Two out of 20 mice, similarly treated, developed sarcomata at the injection site. For the reason stated in the case of CB1348 this result is also indecisive. Fahmy and Fahmy (1953; and personal communication) found that both the D- and L-forms were mutagenic.

The third class consisted of four *purine analogues*. Three of them have been shown to have tumour-inhibitory or antileukaemic effects: 2,6-diaminopurine (2,6-DAP) by Burchenal *et al.* (1949) and Skipper *et al.* (1950); *6-mercaptopurine* (6-MP) by Clarke *et al.* (1953), and Burchenal *et al.* (1953); and *8-azaguanine* (8-AG) by Kidder and Dewey (1949), Gellhorn *et al.* (1950), and Law (1950). It will be recalled that two known tumour-initiators, urethane and TEM (Roe and Salaman, 1955), also possess these properties. 2,6-DAP and 6-MP have both

been shown to induce chromosome abnormalities, the former in mice by Dustin (1950), and the latter in *Allium* and *Pisum* by Kihlman (1952). 8-AG was found by Novick and Szilard (1951) to be mutagenic for *E. coli*.

2,6-*Diamino*-8-*phenylpurine*, a purine analogue recently synthesized by Mr. G. M. Timmis of the Chester Beatty Research Institute, was also tested. Little is known of its biological activity at present.

The fourth class consisted of 10 *miscellaneous* substances, which were chosen for test for a variety of reasons.

Phenylurethane, chemically and pharmacologically related to urethane, had been tested twice before, with a doubtful and a negative result respectively (Roe and Salaman, 1955). The properties of this substance were discussed in that paper. In those tests 0·5 g. and 1·32 g., respectively, were applied. In the test described below a total of 1·8 g. was applied.

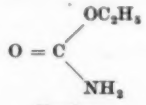
Potassium arsenite, the active constituent of Fowler's solution, was selected because of its antileukaemic action. It was first used in the treatment of leukaemia by Valentiner (1865), and Lissauer (1865), but went out of fashion with the introduction of radiotherapy. Forkner (1938) reintroduced it, and Flory *et al.* (1943) found it efficacious in several strains of mouse leukaemia. Skipper *et al.* (1951) found that potassium arsenite, like urethane, nitrogen mustard, folic acid antagonists, and certain purine analogues (including 2,6-DAP and 8-AG), inhibited the incorporation of labelled formate in the synthesis of nucleic acid purines. Warren (1943) found that arsenite depressed respiration and, probably as a secondary effect, accelerated aerobic glycolysis (urethane also inhibits aerobic glycolysis : Quastel and Wheatley, 1933, 1934). Piton (1929) found that potassium arsenite stimulated mitosis in the intestinal epithelium of the mouse, but that this was followed by death of the mitotic cells. Leitch and Kennaway (1922) painted mice thrice-weekly with a 0·12 per cent solution of potassium arsenite in ethanol, and observed an epithelioma and a benign wart in 1 out of the 30 mice which survived for 3 months or more. The epithelioma had metastasised to the lung. Askanazy (1926) records the development of stomach neoplasms in 4 rats after prolonged administration of potassium arsenite in the drinking water. Cholewa (1935) observed lung tumours in mice given potassium arsenite by mouth, and a sarcoma of the ear in one of two rabbits injected subcutaneously with the substance. Evidence for the carcinogenicity of potassium arsenite, and other arsenical compounds, for both man and experimental animals, is reviewed and discussed by Neubauer (1947).

Three substances, *aspirin*, *saccharin*, and *vanillin*, were chosen for no other reason than that they are commonly ingested. Hartwell (1951) lists four experiments in which aspirin was tested for carcinogenicity in rats ; in three the substance was given by mouth, and in one subcutaneously in lard. No tumours were seen in any of these experiments. Fitzhugh, Nelson, and Frawley (1951) reported an increased incidence of abdominal lymphosarcomata in rats fed for 102 weeks or more on a diet contain 5 per cent saccharin, but the number of animals involved was small (4 with abdominal lymphosarcomata among 18 rats which survived for 1 year or more). Vanillin has apparently not been tested for carcinogenicity.

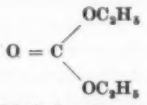
Acrolein, an unsaturated aldehyde ($CH_2 = CH.CHO$), was chosen mainly because it is actively mutagenic for *Drosophila* (Rapoport, 1948). Steiner, Steele, and Koch (1943) injected 15 mice subcutaneously weekly for 24 weeks with

acrolein in sesame oil. There were 11 survivors at 12 months, but no tumours were seen.

Diethyl carbonate was chosen for test because of the similarity of its molecular structure to that of urethane.



Urethane.



Diethyl carbonate.

This substance has apparently not been tested for carcinogenicity on mouse-skin, mouse-lung, or other tissues.

8-*Hydroxyquinoline* was chosen for test on account of its powerful chelating action. No substance with this type of chemical action was included in our previous series. (Salaman and Roe, 1953; Roe and Salaman, 1955).

Monosodium maleic hydrazide was found to exert a growth-inhibitory effect on certain plants by Schoene and Hoffmann (1949), Wittwer and Sharma (1950), and White (1950); and its possible use as a selective herbicide was suggested by experiments of Currier and Crafts (1950). Darlington and McLeish (1951) found that low concentrations of maleic hydrazide ($<0{\cdot}0005$ M) caused chromosome breaks in the roots of *Vicia*; exposure to higher concentrations for 24 hours inhibited mitosis entirely for 2 days. Loveless (1953) tested a series of compounds related to maleic hydrazide (including uracil, thymine, and orotic acid), but failed to observe chromosome-breaks in *Vicia*. Haddow, Koller, and Waymouth (in a private communication to Loveless, 1953) state that they found no evidence that maleic hydrazide gives rise to chromosome-abnormalities in animal tissues, either *in vivo* or *in vitro*. The substance is being tested for carcinogenicity at present (Haddow, personal communication).

Oxalacetic acid was chosen for test because of a report by Rogers (1954), who found that the simultaneous administration of oxalacetic acid with urethane to mice considerably increased the incidence of lung tumours due to the latter. In view of the possible relation between initiating action for mouse skin and lung tumour induction (Roe and Salaman, 1955) it was thought advisable to test oxalacetic acid for initiating action.

MATERIALS AND METHODS

Mice

These were of the "S" strain as used in previous screening experiments. (Salaman and Roe, 1953; Roe and Salaman, 1955).

Chemical substances and solvents

The four substances of Class I 2, 6-diaminopurine, acrolein, oxalacetic acid, and vanillin, were obtained from L. Light and Co. Ltd.

Professor F. Bergel (of the Chester Beatty Research Institute) kindly supplied the three substances of Class II and diethyl carbonate. Professor E. Boyland and Mr. G. M. Timmis (of the same Institute) kindly supplied monosodium maleic hydrazide, and 2,6-diamino-8-phenylpurine and 6-mercaptopurine, respectively.

8-Azaguanine was obtained from Cyanamid Products Ltd., Lederle Laboratories Division.

Aspirin, 8-hydroxyquinoline, ethyl N-phenylcarbamate (phenylurethane), potassium arsenite, and saccharin, were obtained from British Drug Houses, Ltd.

Croton oil.—Two batches (Batches I and II) of oil were used. Both were supplied by Messrs. Stafford Allen. They had been prepared by simple expression of the seeds of *Croton tiglium*, at different dates. Batch II was more irritant than Batch I, and was used at a lower concentration. In other respects the two batches of oil appeared similar (Roe, 1956*b*).

Solvents.—Either acetone (AR grade, British Drug Houses Ltd.) or methanol (James Burrough, Ltd.) was used for all substances, except those of Class III and monosodium maleic hydrazide ; for the latter four substances 50 per cent aqueous carbowax 300 (polyethylene glycol of average molecular weight 300, obtained from General Metallurgical and Chemical Co., Ltd.) was used.

Methods

The techniques of application of solutions, recording of tumours, examination of mice for lung adenomas at post-mortem, and histological examination, have been fully described (Roe and Salaman, 1955).

The choice of dose and of dose-schedule for individual test substances was governed by two factors : solubility in a suitable solvent, and toxicity. Non-toxic substances were applied at the highest concentration obtainable in a suitable solvent, thrice weekly until 10 applications had been given. Toxic substances were applied 10 times at weekly intervals, at the highest tolerated concentration. In all cases (except that of phenylurethane) croton oil treatment was begun between 22 and 30 days after the first application of the test substance.

Further details of the dose-schedules for individual substances are given below and in Table I.

EXPERIMENTAL

Three lots of mice, obtained at different times from the same animal-breeder, were used. From each lot 20 control mice were selected at random to be treated with croton oil only : these will be referred to as control groups A, B, and C, respectively. The remaining mice of each lot were divided into groups of 14 to 25 mice, for treatment with the test substances followed by croton oil.

Treatment with croton oil differed slightly for the three lots of mice. The first lot, including control Group A, received 18 weekly applications of 0·5 per cent Batch I oil (see p. 371). The second batch of mice, including control Group B, received one application of Batch II oil at a concentration of 0·17 per cent ; this caused moderate ulceration in many of the mice, and the concentration of the 2nd and 3rd weekly applications was reduced to 0·085 per cent ; thereafter 0·17 per cent was given and well tolerated for a further 15 weekly applications (making 18 weekly applications in all).

The control group of the third lot of mice, Group C, received 18 weekly applications of 0·17 per cent Batch II croton oil ; but the concentration of the first two applications of croton oil given to the corresponding test groups was 0·1 per cent.

In the case of the less toxic test substances 10 thrice-weekly applications were given at concentrations near saturation in acetone, methanol, or 50 per cent

aqueous carbowax 300. In these groups croton oil treatment was not begun until treatment with the test substance had been completed. Applications of the more toxic substances were separated by weekly intervals, and the earlier applications of croton oil alternated at 3- to 4-day intervals with those of the test substance. Details of treatment are given in Table I. Those tests in which treatment with croton oil overlapped that with the test substance are marked with an asterisk.

Because of the low solubility of anthracene (Group 1) at each of the 10 treatments (thrice weekly) two applications of 0·3 ml. 0·5 per cent were given, separated by an interval of 30 minutes.

The toxicity of the three aromatic nitrogen mustards (Groups 5 and 7) necessitated a close watch on the condition of the mice during treatment. A fall in average body weight was taken as an indication to reduce the dose. Details of dosage are given in Table I.

8-Hydroxyquinoline (Group 15) proved too toxic at the concentration tested (0·5 per cent) for more than 6 weekly applications to be given.

In the case of phenylurethane (Group 18) 2 applications (with an interval of 30 minutes) of 0·3 ml. 20 per cent w/v were given each week for 15 weeks. An interval of 6 weeks followed in which no treatment was given. Thereafter a course of croton oil treatment was begun. This course, it should be noted, was given 17 weeks later than to the corresponding control group (Group A). The long initial course of treatment with phenylurethane alone was intended as a test for carcinogenicity of this substance : it was negative.

RESULTS

(A) *Skin Tumour Production*

Papillomata.—Table I shows the numbers of survivors, of tumour-bearing mice, and of tumours, one week after the end of croton oil treatment.

A few papillomata were seen in two out of three of the control groups treated with croton oil only. In several of the test groups the incidence of these tumours exceeded that in the corresponding control group. In some cases the difference was marked, namely, deoxycholic acid (Group 2), phenanthrene (Group 3), pyrene (Group 4), CB1348 (Group 5), the isomeric phenylalanine mustards (Groups 6 and 7), phenylurethane (Group 18), and saccharin (Group 20). Statistical analysis of the results by the *t*-test, however, showed that only in the cases of CB1348 (Group 5) and phenylurethane (Group 18) are the differences significant : the test for the difference between the mean numbers of tumours per mouse in Groups 5 and C gives $t = 3{\cdot}07$ on 34 degrees of freedom (d.f.), $P < 0{\cdot}01$; that between Groups 18 and A gives $t = 2{\cdot}33$ on 32 d.f., $0{\cdot}05 > P > 0{\cdot}01$.

Malignant tumours.—Shortly after the end of croton oil treatment all mice, except those of Groups B and C and a few papilloma-bearing mice of Groups 5 (6 mice), 6 (3 mice), and 7 (5 mice), were killed and examined *post mortem* for lung adenomata (see below). Mice of Group C continued to receive weekly applications of 0·17 per cent Batch II croton oil ; the subsequent development of further tumours in this Group has been reported elsewhere (Roe, 1956*b* ; Group 5). Mice of Group B, and those of Group 5, 6, and 7 which were not killed at the end of croton oil treatment, were kept without further treatment and examined weekly for the development of malignant tumours of the skin.

So far no definitely malignant tumour has been seen in Group B. However

there has been one tumour which arose as a subcutaneous nodule and later (8 months after the end of croton oil treatment) became ulcerated. A biopsy was taken from this tumour 10 days after ulceration was first observed. Microscopically it appeared to be an epithelioma of low-grade malignancy; it had infiltrated the dermis and extended down to the panniculus carnosus, but at no place was infiltration of this muscle layer seen. The mouse died two weeks after biopsy, further sections of the tumour still showed no infiltration of the muscle layer. This tumour is regarded as "probably malignant" (Roe, 1956*a*).

One of the 5 mice retained from Group 7 (L-phenylalanine nitrogen mustard and croton oil) which was kept under observation developed an undifferentiated carcinoma. This tumour first appeared malignant 3 weeks after the end of croton oil treatment. Histologically it showed penetration of the panniculus carnosus (Fig. 1 and 2) and there was a metastasis in the regional lymph node.

One of the 3 mice retained from Group 6 (D-phenylalanine nitrogen mustard and croton oil) developed a squamous carcinoma 15 weeks after the end of croton oil treatment. Histologically this tumour also showed penetration of the panniculus carnosus.

No malignant tumours have so far arisen in mice treated with CB1348 and croton oil (Group 5).

Observation of the survivors of these groups is being continued.

It is concluded that both CB1348 and phenylurethane are effective initiators of skin tumour formation in the mouse. The fact that other substances (deoxycholic acid, phenanthrene, pyrene, saccharin, and the two isomeric phenylalanine mustards) in conjunction with croton oil gave rise to more papillomata than croton oil only, is of doubtful significance. On the other hand, the fact that a malignant tumour arose in Group 6 and in Group 7 among the very few mice kept for observation, suggests that the two isomeric phenylalanine mustards, if given in higher dosage, might shew a significant tumour-initiating effect. Anthracene, 4 purine analogues, and 8 miscellaneous substances, all gave negative results.

(B) *Histological findings in the skin*

Previously it has been shown that among a wide variety of substances tested there is no correlation between the initiation of skin tumour formation and the production of hyperplasia of the epidermis (Salaman and Roe, 1953; Roe and Salaman, 1955). No correlation is found even within a group of substances related to each other chemically and pharmacologically. For instance, among a limited number of alkylating agents, nitrogen mustard (HN2) and R48 (N,N-di-(2-chloroethyl)-β-naphthylamine) produced a characteristic type of epidermal hyperplasia, but did not initiate tumour formation, TEM (triethylene melamine) produced no recognisable change in the skin, but was an effective initiator, while Myleran (1, 4-dimethane-sulphonoxybutane) was inactive in both respects.

The three aromatic mustards included in the present series were tested for action on the skin. They were applied twice, with one week's interval, at a concentration of 0·33 per cent (i.e. 3 times the highest concentration used in the main experiment), and biopsy specimens of the treated skin were removed three days after the 1st and 2nd applications.

CB1348 (0·33 per cent) caused a slight patchy epidermal hyperplasia, with some cellular enlargement, and reduction in numbers of sebaceous glands. This

effect was similar in character to that due to HN2 (0·2 per cent, Salaman and Roe, 1953), but less in degree. Neither of the two phenylalanine mustards (0·33 per cent) produced any recognisable changes in the skin.

(C) *Incidence of pulmonary adenomata*

All mice except those of Groups B and C, and a few papilloma-bearing mice in Groups 5, 6, and 7, were killed shortly after the end of croton oil treatment and examined *post mortem* for lung adenomata.

Mice of Groups 6 and 7, treated with the *dextro*- and *laevo*-phenylalanine mustards respectively, and croton oil, had a high incidence of lung tumours (47 in 13 out of 18 mice examined, and 55 in 11 out of 13 mice examined, respectively). The highest incidence observed among the other test groups was in Group 5, treated with CB1348 and croton oil (13 in 7 out of 12 examined), and in this case the incidence is only slightly above that seen in untreated mice of the same strain (unpublished data).

Applications of croton oil to the skin are not now believed to increase the incidence of pulmonary tumours in mice (Roe, 1956*b*), though at one time (Roe and Salaman, 1955) it was thought that they might do so. In the present experiment the incidence of lung tumours in mice of Group A, treated with croton oil only, was 17 in 10 out of 17 mice examined.

It is concluded that the high incidence of lung tumours in mice of Groups 6 and 7 resulted from treatment with the phenylalanine nitrogen mustards.

DISCUSSION

The present series of tests for initiating action extends previously reported series (Salaman and Roe, 1953; Roe and Salaman, 1955), using similar methods. The substances chosen for test fall into 4 classes: polycyclic hydrocarbons, aromatic nitrogen mustards, purine analogues, and miscellaneous. The last class contains some commonly used drugs and food additives, and some substances chosen either because of reported doubtful carcinogenicity, or because of their chemical relation to other initiators.

Only two substances, phenylbutyric acid nitrogen mustard (CB1348) and phenylurethane, initiated the development of tumours in significantly higher numbers than in corresponding control groups. In six other cases (deoxycholic acid, phenanthrene, pyrene, the *dextro*- and *laevo*-phenylalanine nitrogen mustards, and saccharin) tumour incidence was higher than in controls, but the differences, though suggestive, are not significant by accepted standards.

There is no reason to abandon the view previously expressed (Roe and Salaman, 1955), that substances which initiate tumour development in the skin are either carcinogenic for both the skin and other tissues (*e.g.* 3, 4-benzpyrene and the other

EXPLANATION OF PLATES

FIG. 1.—Carcinoma arising from the dorsal skin of a mouse treated with L-phenylalanine nitrogen mustard and croton oil (Group 7). The tumour, which first appeared to be malignant 3 weeks after the end of croton oil treatment, has penetrated the panniculus carnosus. [Stain: Haematoxylin and eosin-Biebrich scarlet (Salaman and Gwynn, 1951); × 16.]

FIG. 2.—Higher power view of tumour shown in Fig. 1. Carcinomatous tissue can be seen separating the striated muscle fibres of the panniculus carnosus. [Stain: as Fig. 1; × 350.].

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carcinogenic hydrocarbons) or for tissues other than the skin [*e.g.* urethane (Salaman and Roe, 1955); and TEM (Roe and Salaman, 1955)]. There are borderline cases however. Several substances of very weak or doubtful carcinogencity (*e.g.* 1,2-benzanthracene, see Steiner and Falk, 1951, for review) are nevertheless powerful initiators of skin tumour formation (Graffi *et al.*, 1953; Roe and Salaman, 1955); and others, which show a low grade of initiating activity, are either doubtful or very weak carcinogens (*e.g.* CB1348 : Haddow, personal communication; and methylurethane : Roe and Salaman, 1955; Larsen, 1948), or have so far proved non-carcinogenic (*e.g.* phenylurethane : Roe and Salaman, 1955 and present paper, p. 372). The fact that there are such borderline cases should not lead to the abandonment of the hypothesis that there is a correlation between tumour-initiating activity in skin and carcinogenicity for skin or some other tissue. More evidence is required, and factors of dosage, and rapidity of detoxication and excretion, must be considered, before a decision can be reached on this question.

On the other hand, the suggestion that there is a correlation between initiating action in skin and adenoma production in lung (Roe and Salaman, 1955) has not been supported by the results now recorded. For instance, among the three aromatic nitrogen mustards tested, CB1348 acted as an initiator for the skin but did not significantly increase the incidence of lung adenomas, while both the isomeric phenylalanine mustards markedly increased the incidence of lung adenomas but were ineffective as initiators for the skin. Further, as has been mentioned above, β-propiolactone is an active initiator in skin but does not increase the incidence of lung adenomas.

One of the most interesting findings in previous experiments was that a substance can be a powerful initiator of tumour development in the skin without producing epidermal hyperplasia or any other recognised histological change (*e.g.* urethane : Salaman and Roe, 1953; TEM : Roe and Salaman, 1955). Among the substances included in the present series, of the two which showed definite initiating activity CB1348 caused very slight histological changes [similar in nature to, but very much less marked than, those caused by HN2 or R48, neither of which are initiators (Salaman and Roe, 1953)] and phenylurethane (like the more powerful initiator, urethane) caused no detectable change whatever (Roe and Salaman, 1955).

Phenylurethane produced no tumours when applied alone repeatedly to skin (see p. 372). A corresponding test of CB1348 is not complete. All substances or procedures at present known which "promote" tumour-development [*e.g.* croton oil, iodoacetic acid, chloracetophenone (Gwynn and Salaman, 1953), mechanical trauma (Pullinger, 1945)] produce epidermal hyperplasia, and it therefore seems probable that a complete carcinogen, in contradistinction to an initiator, must have this property.

SUMMARY

1. A further 20 substances have been tested for tumour-initiating action on mouse-skin.

2. Among 3 aromatic nitrogen mustards tested, one, NN-di-(2-chloroethyl)-*p*-aminophenylbutyric acid (CB1348), was found to be an initiator of tumour formation. The two others, *p*-di-(2-chloroethyl)-*L*- and -*D*-phenylalanine, each

gave rise to an incidence of papillomata which was not significantly higher than the control level.

3. Four of the other substances tested, deoxycholic acid, phenanthrene, pyrene, and saccharin, also gave borderline results.

4. Phenylurethane which gave borderline and negative results, respectively, in two previous tests, gave a significantly positive result in a third test.

5. Anthracene, aspirin, vanillin, monosodium maleic hydrazide, and 4 purine analogues, were among the substances which gave negative results.

6. A few papilloma-bearing mice from the groups treated with the 3 aromatic nitrogen mustards, and the whole of a croton oil control group, were kept under observation for 8 months after the end of treatment. A malignant epithelioma appeared in both the *L*- and *D*-phenylalanine nitrogen mustard groups 3 and 15 weeks, respectively, after the end of treatment. No malignant tumours were seen in any other group.

7. The majority of mice were killed and examined for lung adenomata shortly after the end of treatment. Among all the substances tested, only the *dextro*- and *laevo*- phenylalanine mustards increased the incidence of these tumours.

8. The results are discussed.

We are grateful to Professor Bergel for suggesting the use of diethyl carbonate, and the 3 aromatic nitrogen mustards, and to Professor Boyland for suggesting the use of maleic hydrazide and saccharin.

We are indebted to Mr. J. G. Chapman, Miss O. M. Glendenning, Mr. W. J. Milton, Mr. J. A. Rawlings, and Mr. A. L. Stiff for their skilled technical assistance. The expenses of this research were partly defrayed out of a block grant from the British Empire Cancer Campaign.

REFERENCES

ASKANAZY, M.—(1926) *Verh. dtsch. path. Ges.*, **21**, 182.
AUERBACH, C.—(1949) *Biol. Rev.*, **24**, 355.
BACHMANN, W. E., COOK, J. W., DANSI, A., DEWORMS, C. G. M., HASLEWOOD, G. A. D., HEWETT, C. L. AND ROBINSON, A. M.—(1937) *Proc. Roy. Soc.* B, **123**, 343.
BADGER, G. M., COOK, J. W., HEWETT, C. L., KENNAWAY, E. L., KENNAWAY, N. M., MARTIN, R. H. AND ROBINSON, A. M.—(1940) *Ibid.*, **129**, 439.
BARRY, G., COOK, J. W., HASLEWOOD, G. A. D., HEWETT, C. L., HIEGER, I. AND KENNAWAY, E. L.—(1935) *Ibid.*, **117**, 318.
BERGEL, F., BURNOP, V. C. E., LEWIS, G. E., STOCK, J. A., DAVIS, W., ROBERTS, J. J. AND ROSS, W. C. J.—(1954) *Ann. Rep. Brit. Emp. Cancer Campgn.*, **32**, 34.
Idem AND STOCK, J. A.—(1953) *Ibid.*, **31**, 6.—(1954) *J. chem. Soc.*, 2409.
BOYLAND, E. AND BURROWS, H.—(1935) *J. Path. Bact.*, **41**, 231.
BURCHENAL, J. H., BENDICH, A., BROWN, G. B., ELION, G. B., HITCHINGS, G. H., RHOADS, C. P. AND STOCK, C. C.—(1949) *Cancer*, **2**, 119.
Idem, MURPHY, M. L., ELLISON, R. R., SYKES, M. P., TAN, T. C., LEONE, L. A., KARNOFSKY, D. A., CRAVER, L. F., DARGEON, H. W. AND RHOADS, C. P.—(1953) *Blood*, **8**, 965.
CHOLEWA, J.—(1935) *Z. Krebsforsch.*, **41**, 497.
CLARKE, D. A., PHILIPS, F. S., STERNBERG, S. S., STOCK, C. C., ELION, G. B. AND HITCHINGS, G. H.—(1953) *Cancer Res.*, **13**, 593.
COOK, J. W., KENNAWAY, E. L. AND KENNAWAY, N. M.—(1940) *Nature*, **145**, 627.

CURRIER, H. P. AND CRAFTS, A. S.—(1950) *Science*, **111**, 152.
DARLINGTON, C. D. AND MCLEISH, J.—(1951) *Nature*, **167**, 407.
DEMEREC, M.—(1948) *Acta Un. int. Cancr.*, **6**, 247.
DUSTIN, P.—(1950) *C.R. Soc. Biol., Paris*, **144**, 1297.
EVERETT, J. L., ROBERTS, J. J. AND ROSS, W. C. J.—(1953) *J. chem. Soc.*, 2386.
FAHMY, O. G. AND FAHMY, M. J.—(1952) *Ann. Rep. Brit. Emp. Cancer Campgn.*, **30**, 42.—(1953) *Ibid.*, **31**, 30.
FITZHUGH, O. G., NELSON, A. A. AND FRAWLEY, J. P.—(1951) *J. Amer. pharm. Ass.*, **40**, 583.
FLORY, C. M., FURTH, J., SAXTON, J. A. AND REINER, L.—(1943) *Cancer Res.*, **3**, 729.
FORKNER, C. E.—(1938) 'Leukaemia and allied disorders', New York (Macmillan Co.)
FRIEDEWALD, W. F. AND ROUS, P.—(1944) *J. exp. Med.*, **80**, 101.
FURTH, J.—(1953) *Cancer Res.*, **13**, 477.
GALTON, D. A. G., ISRAELS, L. G., NABARRO, J. D. N. AND TILL, M.—(1955) *Brit. med. J.*, ii, 1172.
GELLHORN, A., ENGELMAN, M., SHAPIRO, D., GRAFF, S. AND GILLESPIE, H.—(1950) *Cancer Res.*, **10**, 170.
GOPAL-AYENGAR, A. R.—(1952) *Ann. Rep. Brit. Emp. Cancer Campgn.*, **30**, 55.
GRAFFI, A., VLAMYNCH, E., HOFFMAN, F. AND SCHULTZ, I.—(1953) *Arch. Geschwulstforsch.*, **5**, 110.
GWYNN, R. H. AND SALAMAN, M. H.—(1953) *Brit. J. Cancer*, **7**, 482.
HARTWELL, J. L.—(1951) 'Survey of compounds which have been tested for carcinogenic activity.' 2nd edition. Washington (U.S. Government Printing Office).
JENSEN, K. A., KIRK, I., KØLMARK, G. AND WESTERGAARD, M.—(1951) *Cold Spr. Harb. Symp. quant. Biol.*, **16**, 245.
KENNAWAY, E. L.—(1924*a*) *Brit. med. J.* i, 564.—(1924*b*) *J. industr. Hyg.*, **5**, 462.—(1924*c*) *J. Path. Bact.*, **27**, 233.
Idem AND HIEGER, I.—(1930) *Brit. med. J.*, i, 1044.
KIDDER, G. W. AND DEWEY, V. C.—(1949) *J. biol. Chem.*, **179**, 181.
KIHLMAN, B.—(1952) *Symp. bot. upsaliens.*, **11**, 41. [Quoted by Boyland, E.—(1954) *Pharmacol. Rev.*, **6**, 345.]
KLEIN, M.—(1952) *J. Nat. Cancer Inst.*, **13**, 333.
LARSEN, C. D.—(1948) *Ibid.*, **9**, 35.
LAW, L. W.—(1950) *Cancer Res.*, **10**, 186.
LEITCH, A. AND KENNAWAY, E. L.—(1922) *Brit. med. J.*, ii, 1107.
LISSAUER.—(1865) *Berl. klin. Wschr.*, **2**, 403.
LOVELESS, A.—(1953) *Heredity*, supplement to vol. **6**, 293.
NEUBAUER, O.—(1947) *Brit. J. Cancer*, **1**, 192.
NOVICK, A. AND SZILARD, L.—(1951) *Cold Spr. Harb. Symp. quant. Biol.*, **16**, 337.
PITON, R.—(1929) *Arch. int. Méd exp.*, **5**, 355.
PULLINGER, B. D.—(1945) *J. Path. Bact.* **57**, 477.
QUASTEL, J. H. AND WHEATLEY, A. H. M.—(1933) *Proc. Roy. Soc., B*, **112**, 60.—(1934) *Biochem. J.* **28**, 1521.
RAPOPORT, I. A.—(1948) *Doklady Akad. Nauk. S.S.S.R.*, **61**, 713. [Quoted by Boyland, E.—(1954) *Pharmacol. Rev.*, **6**, 345.]
ROE, F. J. C.—(1956*a*) *Brit. J. Cancer*, **10**,61—(1956*b*) *Ibid.*, **10**, 72
Idem AND GLENDENNING, O. M.—(1956) *Ibid.*, **10**, 357.
Idem AND SALAMAN, M. H.—(1954) *Ibid.*, **8**, 666.—(1955) *Ibid.*, **9**, 177.
ROGERS, S.—(1954) *Proc. Amer. Ass., Cancer Res.*, **1**, 41.
ROSS, W. C. J., DAVIS, W., ROBERTS, J. J. AND EVERETT, J. L.—(1952) *Ann. Rep. Brit. Emp. Cancer Campgn.*, **30**, 25.
SALAMAN, M. H., AND GWYNN, R. H.—(1951) *Brit. J. Cancer*, **5**, 252.
Idem AND ROE, F. J. C.—(1953) *Ibid.*, **7**, 472.
SCHOENE, D. L. AND HOFFMANN, O. L.—(1949) *Science*, **109**, 588.

SKIPPER, H. E., BENNETT, L. L., EDWARDS, P. C., BRYAN, C. E., HUTCHISON, O. S., CHAPMAN, J. B. AND BELL, M.—(1950) *Cancer Res.*, **10**, 166.
Idem, MITCHELL, J. H., BENNETT, L. L., NEWTON, M. A., SIMPSON, L. AND EIDSON, M.—(1951) *Ibid.*, **11**, 145.
STEINER, P. E.—(1955) *Ibid.*, **15**, 632.
Idem AND FALK, H. L.—(1951) *Ibid.*, **11**, 56.
Idem, STEELE, R. AND KOCH, F. C.—(1943) *Ibid*, **3**, 100.
VALENTINER, W.—(1865) *Berl. klin. Wschr.*, **2**, 320.
WALPOLE, A. L., ROBERTS, D. C., ROSE, F. L., HENDRY, J. A. AND HOMER, R. F.—(1954) *Brit. J. Pharmacol.*, **9**, 306.
WARREN, C. O.—(1943) *Amer. J. Physiol.*, **139**, 719.
WHITE, D. G.—(1950) *Science*, **111**, 303.
WITKIN, E. M.—(1947) *Cold Spr. Harb. Symp. quant. Biol.*, **12**, 256.
WITTWER, S. H. AND SHARMA, R. C.—(1950) *Science*, **112**, 597.

RENAL ADENOCARCINOMA INDUCED BY FOWL LEUKAEMIA VIRUS

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Received for publication April 12, 1956.

Though spontaneous carcinomas are very common in the domestic fowl, only a very few have been transmitted, and these with great difficulty and usually for very few passages. It is generally agreed that there is no evidence that they are caused by a virus; the known fowl virus-induced tumours are confined to the sarcoma-leukaemia group. It was therefore with considerable surprise that it was discovered that the virus of avian erythroleukaemia will, under certain conditions, regularly induce kidney carcinomas in the fowl.

EXPERIMENTAL

Virus.—The virus used derives from the ES4 strain of Engelbreth-Holm; as the ES indicates, this produces both erythroleukaemias and sarcomas, the latter especially after intramuscular injection into young hosts. For convenience, it will still be referred to as a leukaemia virus, though it is now clear that this is an imperfect description.

Animals.—All fowls were from the closed population of Brown Leghorns maintained at this Centre. Spontaneous tumours are very rare in this flock, especially in young animals. The kidney tumours to be described appeared only in experiments with the leukaemia virus, and have never been seen in equivalent birds injected with Rous 1 virus, the GRCH/16 chemically-induced non-filtrable fowl sarcoma of Peacock and Peacock (1953) or in normals which were being used at the same time and raised with the leukaemia chicks.

Inoculation.—The kidney tumours were first recognized in experiments on the infectivity of the tissues of birds suffering from leukaemia induced by the virus. Infectivity was being determined by inoculation of decimal dilutions of cells or cell-free extracts of various organs into the left leg of the test chicks. Those which had received active material showed the usual results; a sarcoma may (or may not) develop at the site of injection, and the bird may eventually die of erythroleukaemia with massive infiltrations of the liver and spleen, erythroleukaemia with a profound anaemia, or may show a transient leukaemia followed by recovery. Since the flock is essentially free from leukosis, no resistance due to antibodies transmitted from the hen to the chick via the egg are encountered. It is perhaps for this reason that, contrary to some other workers (e.g. Fagreus, 1954) there is no difficulty in using day-old chicks for titrations of virus activity.

Age of host.—No kidney tumours had been seen in a series of several hundred birds inoculated with the virus at the age of six weeks or more. Further investigations showed that the age limit was even more critical, for only chicks less than

two weeks old at the time of intramuscular injection were found to produce the carcinomas ; with one exception, none over this age ever developed the condition after intramuscular inoculation.

Time interval before appearance.—There was no recognisable change in the kidneys up to about 20 days after inoculation. Later than this, a proportion of the birds which died or were killed with a well-developed and progressive leukaemia had affected kidneys, the most frequent interval being 25–30 days after inoculation. All birds with kidney tumours had a frank leukaemia and usually a sarcoma at the site of injection as well. The kidney changes have not been found in birds showing any marked resistance to the leukaemia action of the virus.

Appearance.—Affected kidneys contain one to several dozen grey circular nodules 1–3 mm. in diameter, sometimes obviously cystic. These usually occur at the periphery of the organ, and both kidneys were usually involved to about the same degree (Fig. 1). Apart from this, the kidneys seemed to be in good condition. Microscopically the appearance of the lesions was characteristic and uniform. The tumours were a cystic papillomatous adenocarcinoma of uniform type (Fig. 2 and 3). Occasionally a simple cystic figure was found, probably an early stage of the development of the condition (Fig. 4). In sections the peripheral occurrence of the tumours was even more obvious. The concomitant leukaemia sometimes produced small groups of malignant blood cells, but otherwise the kidney structure seemed healthy.

General.—Only a proportion of the birds in any one experiment showed the kidney tumours. Given that the age of inoculation was correct the chief difficulty would seem to lie in the relatively few birds which will die of progressive leukaemia after 20 days. The most susceptible are probably already dead, for the first deaths occur about a fortnight after inoculation, and the rest may show some resistance to the virus. Accurate timing of the age of death is difficult, because of the great uncertainty of the activity of any virus preparation and the range of susceptibility of the birds. In a typical experiment, decimal dilutions of material made into groups of four chicks for each dilution and active for five dilutions may yield 2–5 fowls with kidney tumours. Details of a representative experiment are to be found in Table I.

Relation to leukaemia virus.—The kidney tumours were obviously connected with the inoculation of leukaemia virus since, as mentioned above, they were never found in animals used for other experiments or in controls. It might be suggested that they result from contamination with another virus picked up during the work, or were present when the virus was received. Such arguments can only be countered by negative evidence, but are not considered to be at all a likely explanation of the present findings. Firstly, the virus had previously been passed for many generations in older birds without any kidney tumours appearing. A virus present at the beginning would therefore be expected to be diluted out. Tumours of any kind are very rare in the flock (Greenwood, Blyth and Carr, 1948), especially in the young birds used in this work, and no virus-induced ones are believed to exist in it. Kidney tumours are not common even among the few cancers seen (adenomatous areas such as are frequently seen in human kidneys do not seem to occur in the fowl). In any case, all experiments described here were done using as source material the blood, spleen or liver from birds which showed no kidney lesions, sometimes for three or more passages of dilute material. Mere passive transfer therefore seems to be excluded, and everything points to the

TABLE I.—*Intramuscular Inoculation of Cell-free* 10 *per cent Extract of Leukaemia Spleen. Age of Chicks*—8 *days.*

Inoculum diluted.	Leukaemia.	Duration of disease (days).	Kidney section.
$0{\cdot}2 \times 10^{-1}$	+	16	−ve
	+	30	Carcinoma
	+	13	0
	+	23	Carcinoma
$0{\cdot}2 \times 10^{-2}$	+	13	0
	+	18	0
	+	30	Carcinoma
	+	14	0
$0{\cdot}2 \times 10^{-3}$	+	15	0
	+	17	0
	+	23	−ve
	+	13	0
$0{\cdot}2 \times 10^{-4}$	+	40	0
	+	37	−ve
	+	36	−ve
	−	(42)	0
$0{\cdot}2 \times 10^{-5}$	+	40	0
	+	32	−ve
	−	(42)	0
	−	(42)	0
$0{\cdot}2 \times 10^{-6}$	−	(42)	0
	+	28	−ve
	−	(42)	0
	−	(42)	0

All surviving birds were killed at the end of 42 days. Kidneys not examined microscopically are indicated by 0.

leukaemia virus itself as being the cause of the kidney carcinomas. In addition, it was noted that susceptibility to the leukaemia and carcinoma always paralleled ; no case of kidney tumour and recovery from leukaemia was ever encountered, as might be anticipated if two distinct viruses were concerned.

Attempts to increase the frequency of kidney tumours.—Several attempts were made to increase either the frequency or the age of onset of these tumours. Of the methods tried, only direct injection of the virus into the kidney seemed to have any success. Since the kidneys of the fowl lie with the dorsal surfaces embedded in the intertransverse fossae of the fused vertebrae and ilia, direct inoculation is relatively simple ; injections of virus were made into the lower part of the caudal lobe, to avoid any complications with the great blood vessels which are closely applied to the surface of the organ, and the branches of the sciatic nerve which traverse it. This usually produced a small sarcoma infiltrating into the kidney substance and the adjacent muscle, and leukaemia. The earliest kidney tumours were found at 17 days, not very much earlier than before ; but were now present in hosts inoculated at the age of 25 days, and much more frequently. Detailed results of one experiment are given in Table II.

Intravenous injection failed to give any kidney tumours at all, either in chicks aged less than 12 days or in older ones ; but this was most likely due to the fact

TABLE II.

		Duration of condition (days).						
		Up to 16.		17–23.		24 (all birds killed).		
Age at inoc.	No.	Leukaemia only.	Leukaemia + carcinoma.	Leukaemia only.	Leukaemia + carcinoma.	Leukaemia only.	Leukaemia + carcinoma.	Regression.
11	9	5	0	1	1	0	0	2
18	7	3	0	1	2	0	1	0
25	10	3	0	1	2	0	2	2

that none of the injected fowls lived for more than 16 days, all succumbing to generalised leukaemia.

A first attempt to infect kidney cells from very young chicks *in vitro* by mixing them with a suspension of virus and incubating for some hours, and then injecting the result intramuscularly into birds less than 12 days old gave a very good yield in the first experiment but failed completely in two others. It is probable that the amount of virus injected was by chance optimum in the first trial, and not in the others.

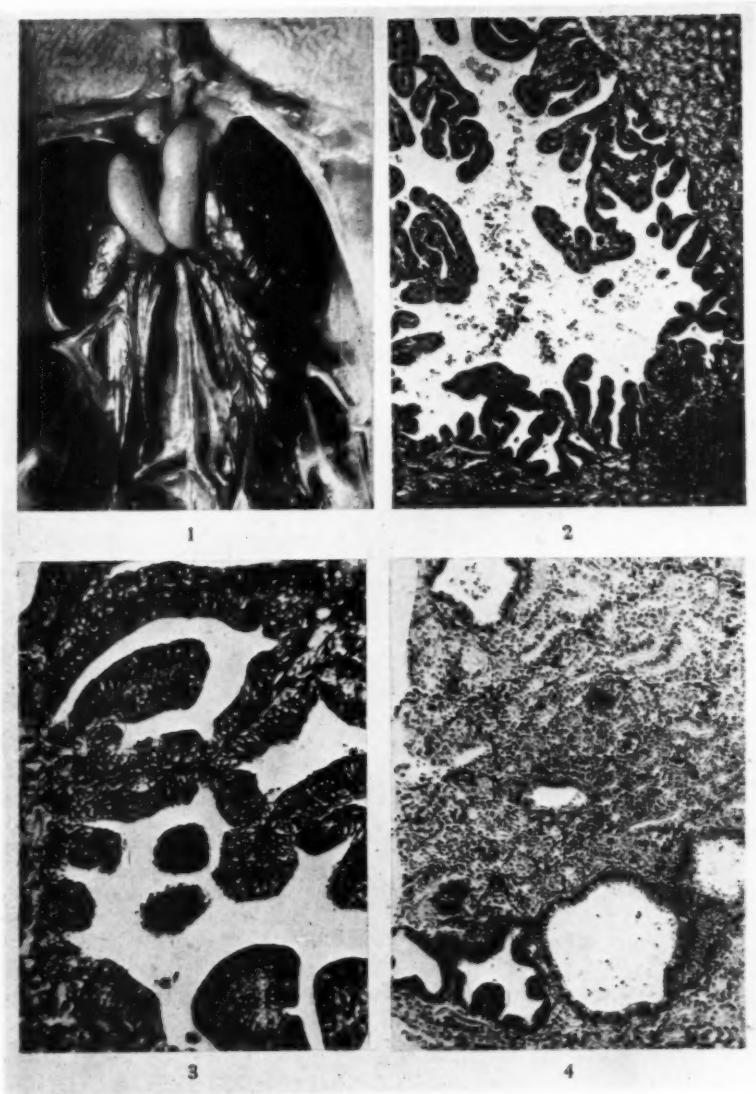
Transplantation attempts.—Since the sections show massive infiltrations of leukaemia blood cells in the kidneys, little hope was entertained that the kidney carcinomas would be successfully transplanted as a pure tumour. This unfortunately proved to be correct, for injection of finely minced kidney from cases with very many carcinomas merely gave the usual sarcoma which results from injection of blood cells or virus, and nothing that could confidently be ascribed to normal or carcinomatous kidney could be found in sections of this growth.

DISCUSSION

The present work clearly indicates that the present conception of the role of viruses in cancer of the fowl requires considerable revision. In the first place, virus-induced carcinomas of the fowl manifestly do exist. The previous restriction to sarcomas and leukaemias had a resulting restriction of ideas on the extent to which viruses could be responsible for the various histological types of cancer; a virus theory which demanded a separate virus for each different histological type of tumour being not generally acceptable. In the case of the best-studied material, that of the domestic fowl, it has been demonstrated that at least one virus can produce a sarcoma, leukaemia, or carcinoma, depending upon the conditions of inoculation. This is analogous with the findings of Gross (1951), who showed that cell-free extracts of mouse leukaemia could induce salivary gland carcinomas when injected into very young susceptible mice. Recent research on cancer-inducing viruses seems to emphasise this wide cytotropism; for example

EXPLANATION OF PLATES

FIG. 1.—Adenocarcinomatous nodules on kidney. Lungs at top, adrenals and testis centre; kidney lobes studded with raised grey cystic nodules.
FIG. 2.—Section of adenocarcinoma; note accumulation of leukaemia cells bottom right.
FIG. 3.—Another adenocarcinoma; higher magnification.
FIG. 4.—Cysts and early adenocarcinomas.



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Duran-Reynals (1947) has shown that the Rous virus can be persuaded by heterotransplantation techniques to give many varieties with new cytotropism and species specificities, such as bone tumours in ducks, while Rose and Rose (1952) by a similar approach were able to obtain bone tumours from the frog kidney virus.

This point is of some interest in connexion with the condition known as the fowl leukosis complex. This consists of an ill-defined array of virus-induced diseases, such as the leukaemias, sarcomas, osteopetrosis, neurolymphomatosis, etc. It has been argued that such different diseases, each with a characteristic age of onset, could hardly be due to a single virus, or closely related group. On the contrary, considering the present results with a leukaemia virus, together with the work on other cancer viruses, this seems quite plausible.

A tentative suggestion may be offered for the involvement of the kidney by the virus. This organ is of mesenchymal origin, and in the chick many embryonic features are still present when the bird hatches. Exclusively adult-type structure is only reached after a few weeks of age. These embryonic parts are concentrated at the edge of the organ, the part where the carcinomas appear. Since viruses have a more extended cytotropism with embryonic cells, the infection of the kidneys is therefore not inexplicable.

The only other virus-induced tumour of the kidneys so far known is that described by Lucké (1934) in the leopard frog, and the similarity of the histological picture of the two conditions is very striking.

SUMMARY

The ES virus of erythroleukaemia of the fowl will also cause adenocarcinoma of the kidney in young fowls, though not in older animals.

All expenses in connection with this work were borne by the British Empire Cancer Campaign.

REFERENCES

Duran-Reynals, F.—(1947) *Cancer Res.*, **7**, 99.
Fagreus, A.—(1954) Ciba Foundation Symposium on Leukaemia Research. London (J. & A. Churchill Ltd.).
Greenwood, A. W., Carr, J. G. and Blyth, J. S. S.—(1948) *Brit. J. Cancer*, **2**, 135.
Gross, L.—(1951) *Proc. Soc. exp. Biol. N.Y.*, **72**, 27.
Lucké, B.—(1934) *Amer. J. Cancer*, **20**, 352.
Peacock, P. R. and Peacock, A.—(1953) *Brit. J. Cancer*, **7**, 120.
Rose, M. S. and Rose, F. C.—(1952) *Cancer Res.*, **12**, 1.

APPARENT PARADOXICAL EFFECTS OF LANOLIN ON INDUCTION OF SKIN AND LUNG TUMOURS BY TOPICALLY APPLIED METHYLCHOLANTHRENE

A PRELIMINARY REPORT

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Received for publication February 7, 1956.

INTEREST in the effects of lanolin on the induction of tumours of the skin by locally applied carcinogens has waned somewhat since the publication of the study by Berenblum and Schoental (1947). These authors reviewed the literature indicating that lanolin has an inhibitory effect on the induction of skin tumours by topically applied methylcholanthrene. They reported, moreover, that the inability to induce skin tumours by the topical application of methylcholanthrene dissolved in lanolin is entirely attributable to the fact that, in such a non-volatile solvent, the effective concentration of the carcinogen is much lower than when dissolved in volatile solvents, such as benzene or acetone. To prove their point of view, Berenblum and Schoental showed that by increasing the concentration of methylcholanthrene (MCh) in lanolin the total tumour yield was " at least as great as in the control group " (treated with MCh or 9,10-dimethyl-1,2-benzanthracene in benzene). They concluded that :—

> " . . . provided the concentration of the carcinogen is high enough, lanolin serves as an exceptionally favourable medium for *facilitating* carcinogenesis. In view of this, its recommended use as a preventive measure against occupational cancer would seem to be of doubtful value. Finally, conclusions regarding the mechanism of carcinogenesis, based on the belief that the inhibitory effect of lanolin is specific, must be deemed invalid in the absence of more substantial supports." (Our italics.)

A justifiable criticism of these results, reported by Berenblum and Schoental, is that although they administered MCh in 3 per cent solution in lanolin, in the one experiment, they failed to conduct a control experiment in which 3 per cent of MCh in benzene was used as well.

Plaut and Sobel (1949) compared the carcinogenicity of MCh when dissolved in benzene, lard, human sebum (from dermoid cysts) and lanolin. Methylcholanthrene dissolved in lanolin failed to induce skin neoplasms, while MCh applied in the equally non-volatile vehicles, lard or sebum, did, albeit more slowly than when benzene was the solvent. Plaut and Sobel consequently concluded that the absence of neoplasms, in mice painted with MCh in lanolin, could not be attributed to the non-volatile character of the lanolin, as claimed by Berenblum and Schoental (1947). Plaut and Sobel having thus failed to confirm Berenblum and Schoental's findings that lanolin had no particular anticarcinogenic actions, suggested that

their results supported earlier claims for some special "anti-carcinogenic" action for lanolin, at least in the mouse.

Piccagli *et al.* (1954) reported that :

> "Interspersed applications of lanolin diminish the total exposure of the skin to methylcholanthrene, while interspersed applications of glycerin or petrolatum increase the persistence of the available carcinogen."

Subsequently, Sulzberger *et al.* (1954) also reported that :

> "A high percentage of mice painted with methylcholanthrene in *lanolin* developed tumours which appeared late, but most of which immediately presented the characteristics of malignancy. This late development of 'carcinoma d'emblée' differs distinctly from the character of tumour formation observed after the application of methylcholanthrene solutions in benzene."

Thus, as far as the action of lanolin in relation to the induction of skin carcinoma is concerned, there now exists considerable doubt as to, firstly, the possible suppressive effect of lanolin on tumour induction and, secondly, the mode of action of this substance on methylcholanthrene absorption and the duration of MCh action on the skin when applied in lanolin.

Lung tumours (primary pulmonary adenomatosis) have also been induced by the topical application of tar (Murphy and Sturm, 1925), or the oral administration and/or intravenous injection of methylcholanthrene (Lorenz and Stewart, 1940 ; Esmarch, 1941 ; Morton and Mider, 1941). Murphy and Sturm (1925), in discussing the possible mode of production of lung tumours by coal tar, when applied repeatedly to different skin fields in rotation, and in the absence of associated skin tumours, considered several possibilities. Firstly, that the lung tumours were metastases—this was excluded by their experimental procedure, in which skin tumours were avoided ; secondly, they entertained the possibility that the lung tumours in their experimental animals treated with tar may have been spontaneous —this was excluded by the fact that although their strain of mice had a high spontaneous incidence of primary lung cancers, the age of their experimental animals was lower than that at which a high incidence of spontaneous primary lung tumours could be ordinarily anticipated ; the third possibility considered by Murphy and Sturm (1925) was "that the particles (of carcinogen) get into the lungs through lymphatics" (following the absorption of the carcinogen from the skin, or from the alimentary tract, if it had been licked off the skin). These authors regarded this possibility as "a little far-fetched", since, they argued, "in either case the tar would have to pass one set of lymph glands in case it was to reach the lungs". Murphy and Sturm (1925) concluded that, since repeated application of tar greatly reduces the resistance of animals to transplanted cancers, that treatment with this carcinogen promoted carcinogenic action of inhaled ordinarily non-carcinogenic but irritating foreign particles of sawdust or wood shavings.

Stewart (1953) reviewed much of the literature relating to the experimental production of primary lung tumours in mice. He states that :

> "The method of intravenous injection is one of the best ways to test exogenous pulmonary carcinogens. Here, the particle size is important, for

> the larger the particles, the more apt they are to be entrapped within the pulmonary capillaries and consequently the greater is the observed carcinogenic effect . . . pulmonary carcinogens act directly upon the pulmonary tissue in which there resides a potential neoplastic process as evidenced by the spontaneous incidence of these tumours. . . . With present methods of qualitative absorption-spectrum analysis it is usually impossible to detect the presence of the carcinogenic hydrocarbons in pulmonary tissue later than one week following its administration."

Hitherto, no light has been shed on the way in which MCh induces primary pulmonary adenomata after application to the skin in volatile solvents.

In the studies reported here, it has been found, albeit in small groups of animals, that the repeated application of lanolin, to the skin of mice that had previously received a known carcinogenic dose of methylcholanthrene, seems to suppress or delay the onset of neoplasia and especially of malignancy in the treated skin, while, at the same time, apparently exerting a slight promotive action on the development of primary lung tumours, apparently induced in our animals by topical application of methylcholanthrene in acetone.

In view of the small number of animals used in the present study, the appended report must be regarded as *essentially preliminary in nature*. However, since previous experiments conducted along identical lines, in our laboratories, have yielded similar results, this preliminary report seems merited at this stage.

MATERIAL AND METHODS

Methylcholanthrene (0·3 per cent in acetone) was applied twice weekly to the sacral skin of 60 albino mice of unknown strain, bred from stock derived from the Onderstepoort Veterinary Laboratories, and initially two months of age. A total of 20 applications of methylcholanthrene were given as one drop with a dropper to each mouse, over a period of 10 weeks. During the early phases (by the third week) of the experiment it was noted that depilation of the MCh-treated area in 13 of the animals was much slower than that in the remainder of the group. These animals were therefore set aside, in a separate cage, and received no further treatment after the twentieth application of MCh. This was done in view of the reported findings by Andreasen (1953) and by Andreasen and Engelbreth-Holm (1953) of the apparent relationship between the stage of the hair cycle at the time of first application of carcinogen, and the subsequent speed of development as well as the incidence of skin tumours in mice. According to the findings of Andreasen and Engelbreth-Holm (1953), animals which depilate slowly after the application of methylcholanthrene are less susceptible to neoplasia in the carcinogen-treated area, and also develop such tumours more slowly. Consequently, this group of " slow depilators " in our experimental series was regarded as an excellent " conservative " control group, from the point of view of the incidence of skin tumours in our mice.

Two weeks after the twentieth application of methylcholanthrene, i.e. at the end of the twelfth week, the remaining 47 mice (i.e. excluding the " slow depilator " controls) were examined clinically and were divided into two main groups, i.e. those which already had papillomata and those which were free of macroscopically detectable benign or malignant tumours. As indicated in Table I, these two major groups were subsequently subdivided still further, so that some received lanolin

applications three times a week, and others had one drop of 95 per cent alcohol applied three times a week to the skin area previously treated with MCh (Table I). The lanolin treatment comprised the application, with a dropper pipette, of one drop of liquid lanolin three times a week to the skin area previously treated with methylcholanthrene. The lanolin was made liquid by immersing the closed container in warm water (40° C.) for some time before use and by keeping the water warm until lanolin application to all animals was completed. Both the lanolin and the alcohol treatments were commenced thirty-three days after the twentieth application of MCh (i.e. the fourteenth week of the experiment) and were continued until the termination of the experiment at the 38th week after the first MCh application.

To determine the spontaneous incidence of lung and other tumours among mice in our colony a further group of 13 mice of the same initial age were permitted to age, untreated, until they were sacrificed when 322 days (46 weeks) old.

Weekly clinical examinations were done on all mice, and the times of onset of benign and/or malignant tumours were recorded for each animal in each group. Full post-mortem examinations were performed on all the mice at the termination of the experiment. Paraffin sections of the MCh- and lanolin- or alcohol-treated skin areas were made in all instances, to determine the state of the skin at the end of the experiment and to assess microscopically the nature of the skin tumours present at death. Lungs were carefully examined in all animals, macroscopically, and any obvious nodules or suspected early pulmonary lesions were sectioned—frequently serially—to determine whether the lung tumours, in each animal, were primary pulmonary adenomata or metastases from malignant skin tumours.

FINDINGS

Skin tumours

In animals which had papillomas at the time lanolin or alcohol treatments were commenced, skin tumours (benign and malignant) developed in almost equal numbers, and at approximately the same rate as in the comparable group of untreated controls. The incidence of malignant tumours in lanolin-treated animals in Group C (Table I) seemed to be lower than in the alcohol-treated group (Group B), but carcinoma occurred with equal frequency in Group A (slow depilators) and Group C.

The most striking differences in the incidence of neoplasia were observed in those groups (D and E) which were devoid of papillomas at the twelfth week of the experiment (Table I). All the animals in Group D developed tumours by the 38th week, and of these animals 63 per cent had microscopically verifiable carcinomas. On the other hand, in Group E, 5 out of 8 animals (62 per cent) failed to develop any tumours at all by the 38th week of the experiment, and the remaining 3 mice in this group had microscopically determined malignant tumours (carcinomas and sarcomas—38 per cent). Among these latter 3 mice the skin tumour in one was diagnosed histologically as a sarcoma, in another a localised small carcinoma lay encapsulated in a large spindle-celled sarcoma while in the third a small ulcer in the skin was flanked on either side by carcinomata which had apparently developed within abnormal cystic hair follicles such as those described by Rogers and Rous (1951) as " pustule cancers ".

TABLE I.—*Treatment and Tumour Incidence in Mice previously receiving 20 applications 0·8 per cent Methylcholanthrene in Acetone to Skin over 10 Weeks.*

Group.	Experimental Groups.	Total mice per group.	Treatment after 14th week.	Skin tumour incidence at 38th week. Nil. No.	Nil. %§	Papillomas.† No.	Papillomas.† %§	Carcinomas. No.	Carcinomas. %§	Primary lung tumour incidence. No.	Primary lung tumour incidence. %§
A	"Slow depilators"* . . .	10	Nil	—	—	2	20	8	80	5	50
B	100% papillomata (at 12th week)	10	95% alcohol 3 × weekly	—	—	—	—	10	100	5	50
C	100% papillomata (at 12th week)	11	Lanolin 3 × weekly	—	—	3	27	8	73	5	45
D	No papillomata (at 12th week) .	8	95% alcohol 3 × weekly	—	—	3	37	5	63	4	50
E	No papillomata (at 12th week) .	8	Lanolin 3 × weekly	5	62	—	—	3	38	6	75
F	Untreated controls‡ . . .	13	Nil	13	100	—	—	—	—	1	8

* "Slow Depilators" implies mice in original series which depilated slowly after the first 3 applications of methylcholanthrene.

† Implies papillomas *only* (unassociated with malignant carcinomata).

‡ This group of mice was untreated throughout the experiment and the animals were sacrified when the same age as the experimental group at the end of the experiment, i.e. when 322 days (46 weeks) old.

§ Percentages are included, not that the authors attach any statistical significance to percentage differences in this small series, but simply to facilitate presentation in the text of data in this table.

Clinical observations revealed that the rate of development of papillomas and subsequently of macroscopically detectable malignant skin tumours was considerably slower in the lanolin treated group (Group E) than in the alcohol treated group (Group D). All skin tumours present at the termination of the experiment were classified microscopically.

Analyses of the times of onset of papillomas and of clinically obvious malignant tumours indicated that the application of lanolin to a skin area which had previously received a known carcinogenic dose of MCh, had apparently delayed the onset and diminished the total incidence of all types of skin tumours and, in particular, had markedly diminished the supervention of malignancy in skin tumours, at least during the time for which these experiments were continued.

These effects of lanolin were especially well marked if lanolin application was commenced before any skin tumours had developed. Lanolin did not seem to exert any marked effects on the incidence of malignant or other tumours if applied after benign tumours had already appeared; however, it did apparently alter somewhat the pathogenesis of the malignant tumours.

Lung tumours

The spontaneous incidence of pulmonary tumours in control untreated mice of our strain and under our experimental conditions was 8 per cent (Table I, Group F).

Among the MCh-treated mice in Group A, 50 per cent had primary lung tumours at the 38th week, while of the total number of 18 mice which were treated with alcohol after the 14th week of the experiment (Groups B and D), 50 per cent

had primary lung tumours at the 38th week of the experiment. Among all the animals receiving lanolin after the 14th week of the experiment (Groups C and E) 69 per cent had primary lung tumours. It is interesting to note that only 45 per cent of the mice in Group C had primary lung tumours, while 75 per cent of the mice in Group E (no papilloma at 12th week and treated with lanolin) developed primary lung adenomata.

It is not possible to state, on the basis of so few animals, whether the differences in the incidence of lung tumours observed in the different groups were significant. However, lanolin treatment certainly did not diminish the incidence of lung adenomata as it seemed to do for the skin tumours and in fact appeared to have a slight promotive effect on the production of lung carcinomas.

DISCUSSION

The experiments recorded here were conducted on such small groups of animals that they do not warrant a detailed discussion. Nevertheless, the results merit recording, even at this stage, in view of the apparently marked suppression of skin neoplasms by lanolin when applied *after* a full carcinogenic dose of methylcholanthrene, but before the appearance of any skin tumours. Although lanolin does not completely suppress neoplasia in MCh-treated skin, it nevertheless does seem to delay markedly the appearance of benign tumours and also seems to retard malignant changes in skin treated with a known carcinogenic dose of MCh. The lanolin, in addition, appears to alter somewhat the pathogenesis of malignant skin tumours as originally indicated by Sulzberger *et al.* (1954). Lanolin, however, seems incapable of suppressing the progressive development of benign skin neoplasms which were present prior to the initiation of lanolin treatment; nor could the supervention of malignancy be completely averted by lanolin treatment.

Although our findings suggest that lanolin alters the reactivity of a MCh-treated skin and somehow suppresses neoplastic tendencies known to be present in such carcinogen-treated areas, the manner in which it produces these effects is obscure.

In the experiments described by Simpson, Carruthers and Cramer (1945), by Berenblum and Schoental (1947), by Piccagli *et al.* (1954), and by Plaut and Sobel (1949) lanolin was applied simultaneously with or shortly (24 hours) after the carcinogen. In these circumstances, lanolin may indeed have altered the "effective" concentration of the carcinogen as suggested by Berenblum and Schoental. On the other hand, the findings of Piccagli *et al.* (quoted above) suggest that lanolin may have acted in these experiments by shortening the duration of action of a previously applied carcinogen simply by promoting its rapid absorption into the lymphatics or even into the blood stream.

According to Simpson and Cramer (1943), Billingham, Orr and Woodhouse (1951) and Piccagli *et al.* (1954), MCh (if applied in volatile solvents) is not detectable, even by sensitive fluorescence-microscopic tests, two or three days after a single application. Since, in our experiments, the lanolin was first applied only 33 days after the last application of MCh, it seems probable that all the previously applied carcinogen had already acted fully and had been absorbed or sloughed with hyperplastic epithelial cells prior to the first lanolin applications. In the light of these findings, as well as those of Plaut and Sobel (1949), the explanation for the "apparent" anticarcinogenic action of lanolin in the experiments assessed,

and also conducted by Berenblum and Schoental (*vide supra*), cannot account for the results in our experiment.

Some mechanism(s) other than that described by Berenblum and Schoental (1947) must be adduced to explain the apparent anti-neoplastic action of lanolin as used in our experiments. The simplest explanation would be that, by its mollifying action, lanolin suppressed scratching of the hyperplastic, carcinogen-treated area by the mice, thereby diminishing the possible co-carcinogenic or " promotive " action of minor traumata. This, of course, is difficult to assess, but would seem to be an unlikely explanation in the light of Berenblum's remarks (1954) that, in the mouse (as opposed to the rabbit) even deep injury, is at best, only a weak promotive stimulus.

Lanolin, in our experiments, may have exerted its seeming anticarcinogenic effects on skin neoplasia through some metabolic action, at least on epidermal cells if not also on the underlying connective tissues. Thus, Mayer (1936) found that the growth-promoting factor in embryo extracts can be inhibited by lipin preparations such as those extractable from mammalian brain by petrol-ether. Kandutsch and Baumann (1954, 1955) demonstrated that the application of a variety of carcinogenic agents produced a rapid and profound drop in the concentration of the fast-acting sterol Δ^7-cholestenol. This drop in skin lipids could not be produced by the application of vitamin A, squalene or oleic acid, even though the latter produced depilation and marked epithelial hyperplasia in mice. Squalene not only failed to produce depilation and a drop in Δ^7-cholestenol concentration, but even induced an increase in this fast-acting sterol in mouse skin. The correlation between decrease in the estimated content of fast-acting sterol in mouse skin and the carcinogenic potency of various compounds was so close as to lead Kandutsch and Baumann to suggest that :

> " . . . the capacity of a substance to reduce the Δ^7-cholestenol concentration of mouse skin might be useful as a rapid preliminary test for skin carcinogens."

In the light of these facts, it is also relevant and interesting to note that, according to Flesch (1951), squalene fails to induce depilation in mice, although it does so in rabbits and guinea-pigs. Flesch suggested that in the latter two species the depilatory and sulphydryl inactivating effects of squalene may be due to its ability to alkylate the sulphydryl groups by virtue of the unsaturated bonds in its molecule ; Flesch also found that squalene inactivates free sulphydryl groups in human epidermis and mouse liver and inhibits succinic dehydrogenase activity of mouse liver. It may be of significance to compare these findings and suggestions by Flesch with those of Kandutsch and Baumann, who confirmed Flesch's finding that squalene fails to depilate mice and added the information that squalene promotes an increase in the Δ^7-cholestenol content of mouse skin. Plaut and Sobel (1949) found that the samples of human (dermoid) sebum tested by them differed from lanolin not only in being a less effective " anti-carcinogen " than lanolin, but also in that the sebum contained squalene and only " traces " of " isocholesterol " whereas the lanolin used by them was free of squalene and contained " isocholesterol ". These latter workers suggested that " . . . the triterpenoids of sebaceous materials may play some role in carcinogenesis or anti-carcinogenesis."

Thus, Mayer (1936) demonstrated that lipids may antagonise certain growth-

promoting factors; Flesch (1951) showed that squalene inhibits or inactivates important intra-cellular enzymes; Plaut and Sobel's (1949) findings indicate that triterpenes may be important in carcinogenesis and anti-carcinogenesis; Kandutsch and Baumann (1954, 1955) revealed a drop in certain skin lipids of mouse skin following treatment with carcinogens and our own observations support the possibility that lanolin may indeed be anti-carcinogenic, at least in the mouse. It seems possible that carcinogens may promote neoplasia by diminishing the normally inhibitory action of skin lipids on the growth and/or mitosis of epidermal cells. The possibility that lanolin may act by rectifying the changes in skin lipid and enzyme concentrations induced by previous or simultaneous treatment with carcinogens certainly indicates that these aspects of the problem would seem to merit closer histological, histochemical and chemical examination. Such studies may throw considerable light not only on the pathogenesis of neoplasia generally, but also on the mode of action of some known carcinogens and anti-carcinogens.

The evidence from this preliminary study (coupled with the findings of Plaut and Sobel, 1949) seems to contra-indicate the view expressed by Berenblum and Schoental (1947) that the recommended use of lanolin as a preventive measure against occupational cancer (Twort and Twort, 1934) "would seem to be of doubtful value". From an essentially practical viewpoint, at least, it seems possible that individuals exposed to intense ultraviolet irradiation and susceptible to "farmer's" or "sailor's" skin, and the almost inevitable neoplastic sequelae thereof, may indeed benefit by the repeated application of lanolin, or of some substance perhaps contained within lanolin and having anti-carcinogenic actions. Such treatments may, in the light of our findings, have very real neoplasia-suppressing effects, especially if, as in the present experiments, they are applied before the onset of ultraviolet irradiation-induced neoplasia. This aspect of the problem is receiving further attention in our laboratories and clinic.

As for the primary lung tumours in our mice—the apparent, albeit still doubtful, slight effects of lanolin in promoting lung tumours induced by topically applied MCh was a coincidental finding in the present study and one which is difficult to explain. Further experiments are presently under way to determine whether or not lanolin, although apparently suppressing skin-neoplasia, may yet perhaps promote lung tumours, simply by facilitating the absorption of MCh in the manner suggested by Piccagli *et al.* (1954) (*vide supra*). Another possibility, also being examined in our laboratories, is that MCh if carried to and/or bound in the lungs by lipids, may exert a more prolonged and therefore greater carcinogenic action on pulmonary tissue.

SUMMARY AND CONCLUSIONS

1. Lanolin was applied to an area of skin previously treated with a known carcinogenic dose of methylcholanthrene (MCh) in acetone. The lanolin was first applied only 33 days after the last of twenty bi-weekly MCh treatments. Analyses of the times of papilloma and carcinoma appearance indicated that, under these circumstances, lanolin seems (*a*) to delay the onset, and (*b*) to diminish the total incidence of all tumours and, particularly, of malignant ones in the skin.

2. These effects of lanolin (on the incidence of MCh-induced skin tumours) are especially well marked if it is applied before any tumours have developed. Lanolin does not seem to exert any noteworthy effects on tumour incidence if it is applied after even benign tumours have appeared.

3. Possible modes of action of lanolin in suppressing skin tumours, in the present experiments, are discussed and special attention is directed to the possibility that lanolin may act metabolically by replacing skin lipids known to be diminished by the application of carcinogens.

4. An incidental finding, in the present preliminary study, confirmed previous observations, by other investigators, that MCh may induce primary pulmonary adenomatosis even after application to the skin only. Treatment of the skin with lanolin, as in the present experiments, did not diminish the incidence of primary lung tumours, and may, in fact, have had a slight co-carcinogenic effect on the induction of lung tumours by topically applied MCh.

5. These apparent suppressive effects of lanolin on skin cancers and possible promotive effects on lung tumours are receiving further attention in our laboratories.

We are particularly grateful to the Schlesinger Organisation for a generous grant which made it possible to conduct and present the above investigations. We also wish to express our thanks to Mrs. Florence Powell for her grant in aid of a Cancer Research Library. Mrs. B. H. Robinow's assistance in obtaining journals, under very difficult circumstances, was invaluable and is gratefully acknowledged. Mrs. H. Herzog's secretarial assistance throughout this investigation is much appreciated. Our thanks are also due to Miss Patricia Low, Mrs. Adele Hart and Miss Phyllis Bilbrough for rendering invaluable technical assistance throughout the conduct of these experiments.

REFERENCES

ANDREASEN, E.—(1953) *Acta path. microbiol. scand.*, **32**, fasc. 1, 157.
Idem AND ENGELBRETH-HOLM, J.—(1953) *Ibid.*, **32**, fasc. 1, 165.
BERENBLUM, I.—(1954) *Acta Un. int. Cancr.*, **10**, 21.
Idem AND SCHOENTAL, R.—(1947) *Cancer Res.*, **7**, 390.
BILLINGHAM, R. E., ORR, J. W. AND WOODHOUSE, D. L.—(1951) *Brit. J. Cancer*, 5, 417.
ESMARCH, O.—(1941) *Det. kgl. Danske. vid. Sel. Biol. Med.*, **16**, 1.
FLESCH, P.—(1951) *Proc. Soc. exp. Biol. N.Y.*, **76**, 801.
KANDUTSCH, A. A. AND BAUMANN, C. A.—(1954) *Cancer Res.*, **14**, 667.—(1955) *Ibid.*, **15**, 128.
LORENZ, E. AND STEWART, H. L.—(1940) *J. nat. Cancer Inst.*, **1**, 17.
MAYER, E.—(1936) *Skand. Arch. Physiol.*, **75**, 1.
MORTON, J. J. AND MIDER, G. B.—(1941) *Cancer Res.*, **1**, 95.
MURPHY, J. B. AND STURM, E.—(1925) *J. exp. Med.*, **42**, 693.
PICCAGLI, R. W., HERRMANN, F., ROTHSTEIN, M. J., SERRI, F. AND FRANK, L.—(1954) *Acta derm.-venereol., Stockh.*, **34**, 216.
PLAUT, A. AND SOBEL, H.—(1949) *Cancer Res.*, **9**, 294.
ROGERS, S. AND ROUS, P.—(1951) *J. exp. Med.*, **93**, 459.
SIMPSON, W. L., CARRUTHERS, C. AND CRAMER, W.—(1945) *Cancer Res.*, **5**, 1.
Idem AND CRAMER, W.—(1943) *Ibid.*, **3**, 515.
STEWART, H. L.—(1953) " Pulmonary Tumors in Mice," Chapter 6. in ' The Physiopathology of Cancer ". New York (Hoeber-Harper).
SULZBERGER, M. B., PICCAGLI, R. W., HERRMANN, F., SERRI, F., FRANK, L. AND ROTHSTEIN, M. J.—(1954) *Acta derm.-venereol., Stockh.*, **34**, 234.
TWORT, C. C. AND TWORT, J. M.—(1934) *Lancet*, i, 286.

ADDENDUM

While this paper was with the Editor initial results of a larger experiment still in progress became available. In this new experiment 50 mice were plucked and MCh treatment of the plucked area was commenced thirty-five days after plucking, i.e. the hair of all mice was in the resting phase. Two weeks after the twenty-second application of MCh lanolin was applied three times weekly, as above, to 25 randomly chosen mice; the remaining 25 mice acted as untreated controls. At the 27th week of the experiment (10th week of lanolin treatment), 11 of the 25 controls had tumours (3 being carcinomas) while 6 of the 25 lanolin-treated mice had papillomas only (no carcinomas). These initial findings are in conformity with the results reported above in that among the lanolin-treated mice the total tumour incidence as well as the carcinoma incidence is lower and the onset of papillomas seems, once more, to be delayed.

ACTIONS OF CORTISONE ON CUTANEOUS AND PULMONARY NEOPLASMS INDUCED IN MICE BY CUTANEOUS APPLICATIONS OF METHYLCHOLANTHRENE

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Received for publication February 7, 1956

THE ability of cortisone to expedite the induction, transplantability and metastasis of various types of epidermal and connective-tissue tumours is still somewhat debatable (see Baserga and Shubik, 1954 ; Ghadially and Green, 1954 ; Piccagli *et al.*, 1954, and Gillman *et al.*, 1955 for critical reviews of relevant literature). There is, however, some evidence that cortisone administration may alter somewhat the pathogenesis of methylcholanthrene-induced skin tumours (Boutwell and Rusch, 1953 ; Gillman *et al.*, 1955).

A careful analysis of the literature indicates that discrepancies in the various reported effects of cortisone on the induction, metastasis and transplantability of tumours are largely attributable, firstly, to differences in the times of initiation of cortisone treatment in relation to the application of carcinogens (Ghadially and Green, 1954) and, secondly, to the type of tumour being investigated (see Baserga and Shubik, 1954 for discussion). Thus, Baserga and Shubik who administered cortisone during the " induction " and " developmental " stages of skin carcinomas found that cortisone inhibited tumour development. Ghadially and Green, on the other hand, found that :

> " Local treatment with cortisone, from the commencement of 9 : 10-dimethyl-1 : 2 benzanthracene painting inhibited papillomata formation in the mouse skin, almost completely. Cortisone also produced this effect when allowed to act during the " developmental " phase of carcinogenesis but was quite ineffective when applied solely during the ' pre-induction ' and ' induction ' phases."

We have previously reported that cortisone fails to suppress epithelial growth or neoplasia if given to wounded rabbits or carcinogen-treated mice before, during and after wounding or methylcholanthrene treatment. However, we did find that cortisone delays, although it does not completely inhibit, tumour formation in mice, and also appears to alter somewhat the pathogenesis of the induced skin carcinomas (Gillman, *et al.*, 1955).

It is now generally conceded that cortisone depresses (but does not completely inhibit) connective-tissue growth following wounding or other injuries (Ragan *et al.*, 1950 ; Baker and Whitaker, 1950 ; Selye, 1953 ; Baxter, Schiller and Whiteside, 1951 ; Gillman and Penn, 1956) and suppresses the reactivity of the reticulo-endothelial system, including its ability to manufacture antibodies (see Thomas, 1955, for original findings and review of literature). In our own skin wound-healing

experiments in rabbits, we have found that the administration of cortisone, before and during the healing of incised or excised wounds, depresses the regenerative capacity of the connective tissues but does not completely suppress them.

In a previous brief report from this laboratory it was indicated that because of the intimate interdependence of epidermis and dermis during healing and tumour genesis (Gillman *et al.*, 1955 *a*, *b*, *c* ; Gillman and Penn, 1956) the effects of cortisone on tumour induction in the skin, reported by us, may be attributable to the ability of this steroid hormone to depress connective-tissue regeneration without simultaneously interfering with the multiplication of epidermal cells. In the light of this view, the findings reported below are of a special interest for our hypothesis, more especially, as will be indicated, the effects of cortisone treatment on tumour genesis in the skin are somewhat different from its effects on primary lung tumours simultaneously induced by topical application of methylcholanthrene (MCh).

MATERIAL AND METHODS

In a series of experiments one drop of 0·3 per cent MCh-solution (in acetone) was applied with a dropper twice weekly to the sacral skin of two month-old albino mice for a total of 20 applications (for details see Gillman, Hathorn and Penn, 1956).

In *Experiment 1*, fourteen days after the last MCh application, the mice were divided into four groups, which received the following treatment :

A. Cortisone—100 mg. in 1 c.c. 95 per cent alcohol B. Alcohol—1 drop 95 per cent	1 drop—thrice weekly—to the previously MCh-treated area.
C. Cortisone—0·25 mg. in aqueous suspending medium D. Aqueous suspending medium alone, 0·25 c.c.	Thrice weekly subcutaneous injections.

In *Experiment 2*, thirty-three days after the last MCh application, the mice were divided into three groups which received the following treatments :

1. Cortisone—100 mg. per c.c. in 95 per cent alcohol 2. Alcohol—95 per cent	1 drop—thrice weekly—to previously MCh-treated area.
3. No treatment after 20th application of MCh (" slow depilators "—see Gillman, Hathorn and Penn, 1956).	

In *Experiment 3* mice received initially 20 acetone applications instead of MCh, and thirty-three days later received thrice weekly cortisone applications to the skin as in Experiment 2.

Experiment 1 was terminated at 231 days, and Experiments 2 and 3 at 266 days after the first MCh application. All mice were killed by ether anaesthesia when moribund, or at the conclusion of the experiments. Full post-mortem examinations were conducted and careful notes were made of the macroscopic appearances of the skin and lungs. Paraffin blocks were prepared of the treated sacral skin in all instances, and of the lungs in each case, and other tissues when deemed necessary. Serial sections were frequently made of lesions in the lungs suspected of being neoplasms.

Finally, a group of untreated stock mice of the same age as those in Experiments 2 and 3, were sacrificed when 266 days old, in order to determine the spontaneous incidence of lung tumours in our strain of mice under our experimental conditions.

RESULTS

Skin tumours

In these experiments it was found that cortisone, administered after completion of MCh treatment, topically in alcohol or by injection in aqueous suspending medium, failed to alter the *microscopically*-determined incidence of skin tumours as compared with the MCh-treated controls receiving alcohol topically or cortisone-free suspending medium by injection. This failure of cortisone to suppress skin tumours was consistently observed whether or not tumours were present at the time cortisone treatment was started. Whether or not the alcohol, used as a solvent for the cortisone, operated as a slight co-carcinogen still remains to be determined. Our findings relating to this aspect of the problem have been outlined elsewhere (Gillman *et al.*, 1955). It may be added also, that, in experiments not hitherto reported in full, cortisone was not found to promote metastasis of macroscopically obvious fungating skin carcinomata, as was apparently the case in the experiments reported by Baserga and Shubik (1954).

Lung tumours

It has long been known that primary pulmonary adenomas can be induced in mice by cutaneous applications of methylcholanthrene or other carcinogens (Murphy and Sturm, 1925 ; Lorenz and Stewart, 1940 ; Morton and Mider, 1941). The mechanism whereby cutaneously applied MCh promotes this type of neoplasm still unknown. Topical application of carcinogens has also recently been shown to expedite urethane-induced pulmonary adenomata (Salaman and Roe, 1953).

A. *Control Groups.*—Twenty-eight mice in all the experiments were found to have tumours of the lung. In two animals, microscopic examination revealed the lung tumours to be squamous epitheliomata, and since these were found in mice bearing large, locally infiltrating skin carcinomas, they were classified as metastases (one each in the topical cortisone and topical alcohol groups).

The lung tumours in the remaining 26 experimental mice, together with those occuring spontaneously in the one untreated stock mouse of the same age as the experimental groups, were regarded as typical primary neoplasms, with microscopic appearances distinct from those of the secondary carcinomas. On the basis of both the macroscopic and microscopic criteria detailed by Grady and Stewart (1940) these tumours were classified as primary pulmonary adenomata.

TABLE I.—*Types of Skin Lesions associated with Primary Lung Tumours*

Type of skin lesion.	Number of mice with primary lung tumours.
Carcinoma	13
Carcinoma and sarcoma	2
Sarcoma	3
Papillomas only	3
Without skin lesions	5
Total	26

The condition of the skin of the mice bearing these primary pulmonary tumours is shown in Table I. From this table it will be seen that 5 mice with lung tumours

had no detectable skin lesions at all, and that a further 3 mice had only benign skin lesions. This tends to substantiate our opinion that the lung tumours in our mice were indeed primary pulmonary adenomata.

TABLE II.—*Incidence of Primary Lung Neoplasms*

Experiment No.	Intitial treatment.	Subsequent treatment.	Incidence of primary lung tumours. At 231 days. No.	%	At 266 days No.	%
Stock mice	Nil	Nil	—	—	1/13	8
3	Acetone	Cortisone applications	—	—	0/7	0
2	20 bi-weekly applications of methylcholanthrene (0·3% in acetone)	Nil	—	—	5/10	50
1, 2		Alcohol applications	3/8	37	8/15	53
1, 2		Cortisone applications	0/7	0	6/18	33
1		Aq. susp. med. injections	4/7	57	—	—
1		Cortisone injections	0/5	0	—	—

B. *Effect of cortisone on incidence of primary lung tumours.*—From Table II it will be seen that in the experiments terminated at 231 days, cortisone, whether administered by injection or by topical application appeared to suppress completely the development of primary lung tumours. At this time no tumours were found in any of the 12 cortisone treated mice as compared with 7 out of 15 of the controls.

However, when the findings in Experiment 2 (terminated at 266 days) are considered it is at once apparent that cortisone did not entirely prevent the development of these tumours. However, cortisone did seem significantly to delay their onset to beyond the 231st day of the experiments. The fact that the experiment was not continued for longer than 266 days may alone account for the lower incidence of lung tumours in the cortisone treated mice, even in the second experiment.

Whether or not cortisone, in addition to delaying their onset, would diminish the ultimate incidence of lung tumours if the experiments had been continued for much longer can only be determined by experiments of even longer duration than those reported upon here (see below).

DISCUSSION

In the present experiments cortisone was initially applied only during the " developmental " phase of carcinogenesis, and well after the " induction phase " had been completed. Our findings in the skins of our mice are, therefore, not in accordance with those reported by Ghadially and Green (1954), nor with those of Baker and Whitaker (1949), Baserga and Shubik (1954) or Engelbreth-Holm and Asboe-Hansen (1953). However, our observations do support those of Rusch (1953) and of Boutwell and Rusch (1953). Nevertheless, it should be carefully noted, since it merits such special attention, that all the investigators who reported that cortisone suppresses experimentally-induced skin neoplasia, conducted their experiments for only relatively short periods, ranging from 21 days (Baker and Whitaker, 1949) to a maximum of 26 weeks (Piccagli *et al.*, 1954), as opposed to the experiments reported on by Boutwell and Rusch, and by ourselves, which extended over very much longer periods (up to 33 and 38 weeks in our own experiments).

As indicated above, there is ample evidence in the literature to demonstrate that although cortisone retards connective-tissue regeneration, healing of wounds is, nevertheless, ultimately satisfactorily achieved even in cortisone-treated subjects (human and animal). We have already noted elsewhere (Gillman *et al.*, 1955) that cortisone suppresses and alters the dermal reactions usually encountered in mice following hair plucking or those associated with the epidermal neoplasia induced by MCh. However, epidermal malignancy ultimately occurred in our mice, albeit the pathogenesis of these tumours was apparently altered by the cortisone treatment administered topically or by subcutaneous injection. These earlier observations from our laboratory are confirmed by the presently reported experiments, in respect of the skin tumours. We may also add that in our mice, both in the present and in several other series of experiments (some reported on in the literature and others not), we have not been able to confirm the finding of Baserga and Shubik (1954) that cortisone treatment promotes metastasis of fungating methylcholanthrene induced skin carcinomas.

Regarding the primary pulmonary tumours which were encountered incidentally in our mice—once more our findings indicate that cortisone delays but does not entirely suppress the development of these tumours. Had we studied only animals killed at 231 days of the experiment then it would certainly have seemed that cortisone treatment had completely suppressed the induction of primary pulmonary tumours by cutaneous applications of MCh. However, the results of a subsequent experiment, of longer duration, indicated that cortisone does not completely suppress primary pulmonary adenomatosis, but rather significantly delays the onset of such tumours, which nevertheless appear in greater numbers than among control completely untreated stock mice of similar ages.

Cortisone has been shown by several investigators, including ourselves, to be incapable of suppressing regeneration of the epidermis during wound healing (Lattes *et al.*, 1953 ; Gillman *et al.*, 1955). However, it is generally agreed (see above) that cortisone delays connective-tissue regeneration in healing or inflammation and is apparently not as inhibitory to the growth of transplanted connective-tissue tumours as to epithelial tumours (Baserga and Shubik, 1954).

Like other investigators (Orr, 1939 ; Marchant and Orr, 1953 ; Vernoni, 1951) we have repeatedly drawn attention to the possible role of the connective tissues in epidermal neoplasia (Gillman *et al.*, 1955). It seems possible, at least, that cortisone delays epithelial tumour formation and growth indirectly via its effects on the associated connective tissues. This may explain the retardation of tumour induction, both in the skin and in the lungs of our MCh-treated mice receiving cortisone.

Another explanation may be suggested to account for the apparently more marked (or more prolonged) retarding action of cortisone on tumour induction in the lung as compared with the skin. As shown by Stewart (1953) metastases of primary pulmonary adenomata often exhibit sarcomatous patterns of growth. Moreover, as finally established by Stewart, Grady and Andervont (1947) these tumours frequently undergo partial or complete sarcomatous transformations on serial transplantation. To the four theories advanced by Stewart (1953) to account for this remarkable change in these apparently glandular epithelial lung tumours, yet another possiblity might be added. This fifth theoretical explanation is that the primary lung tumours arise from cells which are derived, embryologically, from mesenchyme. If this ultimately proves to be the case, then it might be expected,

in view of the special suppressive effects of cortisone on other mesenchymal derivatives, that the development of lung tumours would also be markedly inhibited by this hormone. This possibility may also explain the sarcomatous transformation of these tumours on transplantation.

Our findings relating to the effects of cortisone on the skin and lung tumour induction provide a basis for suggesting that more intensive study of the role of connective tissues in the histogenesis of neoplasms is merited. It also seems reasonable to suggest that, by using cortisone, it may yet prove possible to illuminate several aspects of the origins, growth and powers of differentiation of metastases and/or transplants of primary pulmonary adenomas in mice.

SUMMARY AND CONCLUSIONS

1. The effects of topically applied and of injected cortisone on the induction of skin and primary lung tumours by methylcholanthrene have been examined.

2. Cortisone administered in the present experiments only during the "developmental phase" of carcinogenesis, delayed but did not completely suppress the appearance of skin and lung tumours. The retarding effects of cortisone on tumour induction, following topically applied MCh, was more marked in the case of the lung than the skin.

3. Discrepancies in the literature, relating to the action of cortisone on tumour induction are reviewed and attributed: (*a*) to differences in the time of initiation of cortisone administration relative to treatment with carcinogen, and (*b*) to the fact that in many instances the full effects of cortisone on tumorigenesis could not be assessed because the experiments reported on by others were not conducted for long enough.

4. Comparisons have been drawn between the action of cortisone on epithelium and on connective tissue during wound healing, hair growth and tumorigenesis. The conclusion was drawn that the ability of cortisone to retard tumour induction may be due indirectly to the depressive effects of this hormone on the connective tissues. Some possible implications of this conclusion are discussed for understanding tumorigenesis in general, and in particular, the origin and the peculiarities of the growth of metastases and transplants of primary pulmonary adenomas.

REFERENCES

BAKER, B. L. AND WHITAKER, W. L.—(1949) *Univ. Hosp. Bull. Mich.*, **15**, 4.—(1950) *Endocrinology*, **46**, 544.

BASERGA, R. AND SHUBIK, P.—(1954) *Cancer Res.*, **14**, 12.

BAXTER, H., SCHILLER, C. AND WHITESIDE, J. H.—(1951) *Plast. reconstr. Surg.*, **7**, 85.

BOUTWELL, R. K. AND RUSCH, H. P.—(1953) *Cancer Res.*, **1**, 9.

ENGELBRETH-HOLM, J. AND ASBOE-HANSEN, G.—(1953) *Acta path. microbiol. scand.* **32**, 560.

GHADIALLY, F. N. AND GREEN, H. N.—(1954) *Brit. J. Cancer*, **8**, 291.

GILLMAN, T., HATHORN, M., AND PENN, J.—(1956) *Ibid.*, **10**, 384.

Idem AND PENN, J.—(1956) *Medical Proceedings*, Supplement to Vol. 2, 93–186, March, 1956.

Idem, PENN, J., BRONKS, D. AND ROUX, M.—(1955*a*) *Brit. J. Cancer*, **9**, 272.—(1955*b*) *Nature* **176**, 932.—(1955*c*) *Experientia*, **11**, 493.

GRADY, H. G. AND STEWART, H. L.—(1940) *Amer. J. Path.*, **16**, 417.

LATTES, R., BLUNT, J. W., ROSE, H. M., JESSAR, R. A., VAILLANCOURT, G. DE AND RAGAN, CH.—(1953) *Ibid.*, **29**, 1.
LORENZ, E. AND STEWART, H. L.—(1940) *J. nat. Cancer Inst.*, **1**, 17.
MARCHANT, J. AND ORR, J. W.—(1953) *Brit. J. Cancer*, **7**, 329.
MORTON, J. J. AND MIDER, G. B.—(1941) *Cancer Res.*, **1**, 95.
MURPHY, J. B. AND STURM, E.—(1925) *J. exp. Med.*, **42**, 693.
ORR, J. W.—(1939) *J. Path. Bact.*, **49**, 157, 495.
PICCAGLI, R. W., HERRMANN, F., FRANK, L., ROTHSTEIN, M. J., MORRILL, S. D. AND SULZBERGER, M. B.—(1954) *J. invest. Derm.*, **22**, 317.
RAGAN, C., HOWES, E. L., PLOTZ, C. M., MEYER, K., BLUNT, W. J. AND LATTES, R. (1950) *Bull. N.Y. Acad. Med.*, **26**, 251.
RUSCH, H. P.—(1953) *Proc. Amer. Ass. Cancer Res.*, **1**, 5.
SALAMAN, M. H. AND ROE, F. J C.—(1953) *Brit. J. Cancer*, **7**, 472.
SELYE, H.—(1953) *J. Amer. med. Ass.*, **152**, 1207.
STEWART, H. L.—(1953) " Pulmonary Tumors in Mice ", Chapter 6, in " The Physiopathology of Cancer ". New York (Hoeber-Harper).
Idem, GRADY, H. G. AND ANDERVONT, H. B.—(1947) *J. nat. Cancer Inst.*, **7**, 207.
THOMAS, L.—(1955) *Bull. N.Y. Acad. Med.*, **31**, 485.
VERNONI, G.—(1951) *Sci. med. ital.*, **2**, 373.